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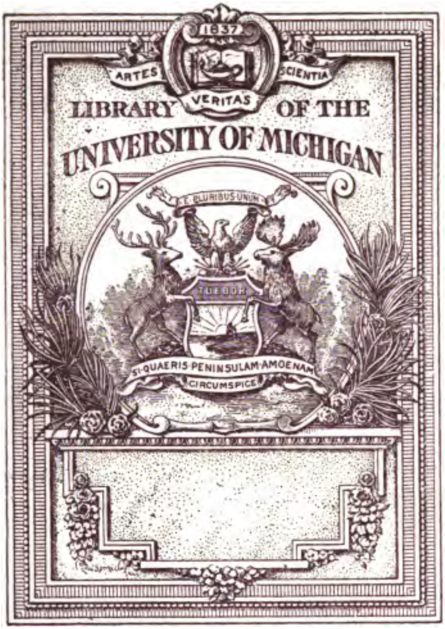

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A COURSE

OF

PRACTICAL ELEMENTARY BIOLOGY

BY

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NEW YORK
LONGMANS, GREEN, & CO.
15 EAST 16th STREET
1893

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PREFACE

THE types described in the following pages have been selected as fairly representing, in so far as they can be represented in a book of this size, the vegetable and animal worlds. It is possible that a better selection might be made, but not one, I think, of which the examples can be more conveniently procured, cultivated, and examined, whilst retaining their capability of teaching so much. The subjects dealt with cover most elementary biological courses, but apparently do not exactly fit any. This, perhaps, is not a disadvantage.

A few words of explanation as to two of the examples may not be out of place. Although it has been said that Chara is "typical only of itself," it seems a pity to exclude it from a general scheme of representative forms when it is remembered with what facility it lends itself to examination, how much can be learnt from it, and to what an extent modern botany is indebted to careful study of the Characeæ. It might almost be said that what the frog has been to the zoologist, the Characeæ have been to the botanist. The selection of a single example of the flowering plant is, I am aware, opposed to common practice; but where the object is to teach, not merely the form and structure of all possible organs, but structure and function, and the correlation of the one with the other, the reason for the study of one example, and that one Lamium, will be evident. He will know a good deal of botany who knows Chara and Lamium thoroughly.

It appears to be a bold matter to recommend the student to commence the study of Biology with the simplest forms. It is said that he should proceed from the known to the

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unknown, and should therefore commence the study of the vegetable side of the science with the flowering plants, and the animal side with the vertebrates. Setting aside the undoubted fact that the flowering plant is, to the beginner, as unknown botanically as yeast, and the frog, zoologically, as amœba, it is also a correct principle that the beginner should proceed from the simple to the complex, and that course is followed here. It is true that the simplest forms are also among the smallest, and beginning at this end necessitates some facility in the use of the microscope. The biologist must, however, like any other workman, commence by learning the use of his tools.

Many of the illustrations are new and have been drawn for this book. I desire to express my great obligation to those publishers and authors who have allowed me to use figures from their works. In all cases the source of a figure is stated in its inscription, and a list of these works, with others, will be found at the end of the introductory chapter.

J. B.

GATESHEAD-ON-TYNE,
November, 1892.

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PRACTICAL ELEMENTARY BIOLOGY.

INTRODUCTION.

To obtain a complete knowledge of the habits, forms, and internal structure of plants and animals, a threefold examination is necessary; first, with the naked eye; secondly, by the aid of some form of dissecting microscope magnifying a few diameters; thirdly, with a compound microscope magnifying from about fifty up to several hundred diameters.

The living organism, whether plant or animal, must be studied in its natural home. Specimens must also be kept at hand under as natural conditions as possible, and closely and continuously observed, but this alone will not be sufficient. Observation in the field must check observation in the laboratory, or faulty views will be formed and wrong conclusions arrived at. In the case of the smaller organisms which are inhabitants of water, amongst a host of observations to be carefully recorded, it must be noted whether they live solitarily or in numbers, whether they inhabit the depths or the surface, whether they love the light or shrink from it. The temperature and hardness of the water, and the force or absence of current, are also important points; and just as important is it to note the shape and size of the apparently shapeless masses, the floating scums, or the matted growths which they make. They, as well as the larger forms, must be sought for at all seasons of the year, for there are frequently wide differences between their forms and habits in winter and in summer, differences which do not appear in laboratory-kept specimens. Even those which apparently die away in winter necessarily leave some representative which retains vitality enough to reproduce the species in spring.

Observation in the field of the larger plants and animals must be as close and continuous as that of the smaller. In studying the larger plants, attention must be paid to the soil, whether hard or soft, wet or dry, cultivated or uncultivated; whether the situation be exposed or sheltered; the time of first appearance, flowering, and setting of the seed; the methods of fertilization and distribution of the seed; and a multitude of other things. In the case of the larger animals, their methods of taking and ingesting their food; the manner of their locomotion; the nature of their habitat; the method of fertilization; the care of their eggs and young, are only a few of the things to be studied.

It is indeed of the first importance that field work should precede and accompany laboratory work. The completest knowledge of structure can never compensate for the absence of that living acquaintance with the plant or animal under natural conditions which is to be got only in the field, and which, by enabling us to correlate its structure with its habits, completes our knowledge of it and solves many a problem which details of structure set before us.

The specimens which are kept in or near the laboratory should be under as natural conditions as possible. For water plants and animals inverted wide-mouthed bell-jars, such as are commonly used for covering delicate plants, form sufficient aquaria. About an inch of silver sand mixed with a little well-washed field soil should be placed at the bottom, and if possible some of the water in which it lives should be removed with the organism and placed in the jar. As the water evaporates, it will be sufficient to fill up with distilled water if plants and animals are kept in the same aquarium. As a rule, the aquarium should stand in a window, but the amount of light entering it can be controlled by pasting translucent or even opaque paper on parts of it. Here the smaller plants and animals may be kept under close observation for a practically unlimited period, with the additional advantage that material for dissection is always available.

The larger plants should also be kept growing close at hand, in their native soil if possible, if not, in as close an imitation of it as can be made. It is obviously more difficult to keep the larger animals under natural conditions.

When specimens are taken for keeping alive, a few in various stages of development should be placed in a preserving fluid of some kind. The most common, and, on the whole, the best for the larger organisms, is alcohol. Methylated spirit, in wide-mouthed stoppered bottles, will do very well, care being taken to use a relatively large amount of the spirit. Characeæ and algæ should first be placed in a concentrated solution of picric acid and then into spirit. The smallest organisms should be mounted as permanent microscopical preparations.

The examination into the structure of the individual should be commenced with the aid of a magnifying-glass of some kind. For very simple work a watchmaker's eye-glass, costing a few pence, is not to be despised. If the student cannot hold it in his eye, he should bore two holes through the horn tube with a hot wire and fasten on a piece of elastic long enough to go round his head and keep the glass in position in front of one eye. The advantage of this is that both hands are left free, and the object may be held up in various positions to be examined. Small tripod doublets are sold for a few shillings. They may be got magnifying four or five diameters, and giving a working distance beneath them of an inch or more, but they can of course be used only on a table or other support. For the beginner, this is good enough; but he will not have advanced far before he will feel the necessity for a better instrument for dissections.

A dissecting microscope should stand steadily on a broad base, it should have a large glass stage, slightly hollowed in the middle, for supporting the object during dissection in water, or other liquid, if necessary, a mirror for reflecting light up through the stage, and lenses magnifying from ten to thirty diameters, or even more. A rack-and-pinion movement must be supplied to bring the object into focus, and the instrument should have arm-rests for supporting the hands whilst working.

The catalogues of most dealers describe dissecting microscopes fulfilling these conditions with the exception of arm-rests, a curious omission, seeing how universally their use is recognized.

Additional apparatus necessary for dissecting plants and animals are—

Two or three scalpels, one of which should be rather blunt for scraping.

Needles of various sizes mounted in wooden handles. One or two should be rubbed down on a hone to flat sharp points.

Small scissors, one with straight and one with curved points.

A pair of forceps with fine points, and a larger pair for handling larger objects.

A few small brushes, some stiff and some soft.

Watch-glasses which will stand steadily, and small white porcelain pots with lids.

A blow-pipe of glass or metal, and a seeker.

Dipping tubes of glass drawn to a point at one end. Over the other should be drawn a rubber nipple without a hole at its free end. These can be obtained from any chemist. To use the tube squeeze the nipple and place the point of the tube in the liquid close to the small object it is intended to remove. Then release the rubber, and the object will be sucked into the tube.

A dissecting dish of white earthenware or glass, not less than 8 ins. × 6 ins. × 3 ins. in size. This will be used mainly for animals. Into the bottom should be run half an inch of melted paraffin weighted with a few pieces of lead to hold it down, or a sheet of cork, or even a piece of soft deal with sheet-lead nailed to its under surface, will serve. Into this pins may be thrust, and by this means the subject of dissection may be firmly fixed underneath the water.

A much smaller dish for smaller objects is also very desirable.

Fresh material will, as a rule, be used for dissection, and a few fresh specimens of each animal must be dissected. But for many purposes animals which have lain in alcohol some days are to be preferred. The alcohol dehydrates the tissues, and thus hardens them. The dissection of an animal is accomplished by removing the connective tissue which holds the organs together. In the fresh state this is transparent, and in many cases difficult to see; but when dehydrated by alcohol it is readily seen.

In dissecting animals the water will soon become discoloured and clouded. When this is the case, it must be immediately changed. Local cloudiness during dissection may be dissipated by directing a gentle stream of water on the spot with the pipette already described.

The pins used should be long enough for their heads to project from the water, but not so long as to be in the way. It is best to insert them obliquely with their heads turned from the subject.

Besides exhaustive dissections of plants and animals, the relations of parts to each other should be studied in sections, both longitudinal and transverse, of the whole organism. The tissues of plants are usually firm enough to allow good sections to be obtained; those which are not may be hardened in alcohol. For such sections animals should be kept in a quarter per cent. solution of chromic acid for some days. This will harden them so that their softest parts will bear a clean cut.

The student must not expect that one or a few specimens of each type will be sufficient for him to acquire a good knowledge of its structure. He will certainly make some mistakes, and each mistake will involve the spoiling of material.

Everything exposed by dissection should be drawn. Elaborate shaded drawings are not necessary, but simple outline sketches should be made; and a great number of simple drawings, each showing a few details, are far better than a few crowded ones. The making of such drawings serves two purposes. In the first place, they help to fix the facts in the mind, in the second place a glance at the drawing will at any time be almost equivalent to a new dissection.

No one can hope to intelligently use a modern compound microscope without some knowledge of its principle. Therefore, before proceeding to the description, methods of manipulation, and of preparing objects for the microscope, it is thought best to give here a short and simple explanation of its principle. For a complete explanation the student is referred to works on general optics and on the microscope.

The light by which we see a non-luminous object is the light reflected from it or transmitted through it. We can also distinguish the outline of an opaque object by reason of the absence of that light which it cannot transmit. In passing through transparent objects some light is always absorbed. Such an object is visible in direct light because it transmits less light than the more perfectly transparent media beside it.

In passing through homogeneous media rays of light follow straight lines; but when they pass from one body to another of different density they usually no longer follow straight lines, but are refracted, or bent aside.

A lens is an instrument for refracting rays of light in a certain definite manner. The following description is confined to glass lenses which are thicker in the middle than at the margins, and whose surfaces are segments of spheres.

Thus let B E (Fig. 1) represent a section of a double-convex lens, C and F centres of the spheres of which its convex surfaces are segments, and C D and F B radii of the spheres. Then any ray of light, L B, which meets the lens at B, is bent towards the radius F B in the lens, and away from the radius C D on leaving the lens, and is therefore refracted twice in the same direction. All the other parallel rays which meet the lens, with the exception of M N, which courses along the radii C A, N F, are bent towards each other, and all meet in the centre F, which is called the principal focus of the lens. F will be a very bright spot of light.

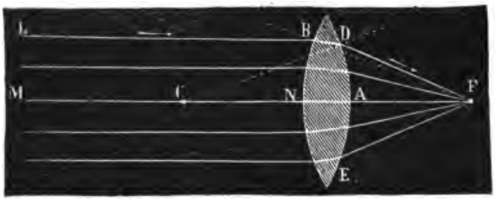

FIG. 1.

Conversely, if a bright source of light be placed at F, the rays which spread from it, and meet the lens, will emerge as parallel rays.

Now, if the source of light be at L (Fig. 2), outside the principal focus, but not at a very great distance, it can be shown that they will be concentrated on the other side of the lens at *l*, which is also beyond the principal focus.

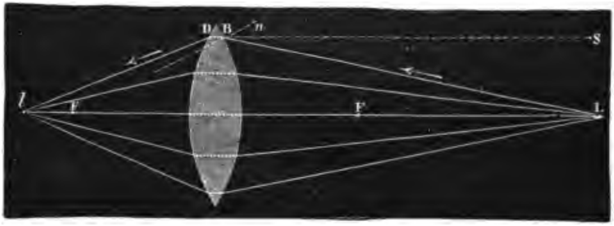

FIG. 2.

But if the source of light be at L (Fig. 3), inside the principal focus, then, on emerging from the lens, the rays will be divergent. They, therefore, do not meet at all on that side. The eye, however, refers an object to the place whence the rays of light from it appear to come. If these divergent rays be received in the eye, they appear to proceed from *l*, and at *l*, therefore, the spot of light appears to be. *l* is not a real but a virtual focus.

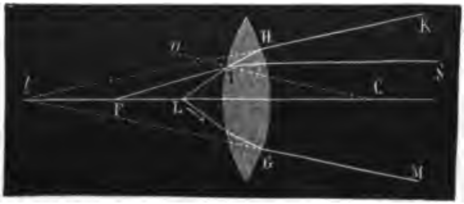

FIG. 3.

Now, applying these principles to the images of objects of definite size, we see that if A B (Fig. 4) be an object a short distance outside the principal focus of a lens, the rays of light from the extremity A, after refraction by the lens, intersect at *a*, and, therefore, the image of the point A is at *a*. Also the image of the point B is at *b*, and the images of all points between A and B must be between *a* and *b*. The image of the whole object A B is therefore *a b*. It is a real image, inverted, however, and in this case smaller than the object.

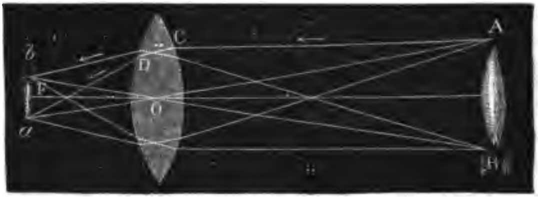

FIG. 4.

If *a b* were the object, A B would be the image, and in that case the image would be larger than the object.

But if the object be placed inside the principal focus, as in Fig. 5, then the rays of light from A, after refraction, will be divergent. They may, however, be received in the eye, and then they appear to proceed from *a*. Also the rays of light from B are divergent, and appear to come from *b*. And all the rays of light from every point of the object between A and B appear to come from points between *a* and *b*. The image of A B is formed, therefore, at *a b*. It is a virtual image, erect, and larger than the object.

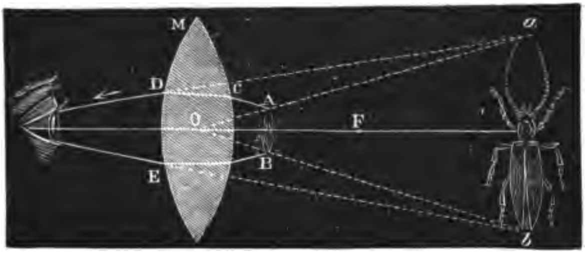

FIG. 5.

It follows, therefore, that in order that a simple lens may be used as a magnifier, it must be placed at a shorter distance from the object than its own principal focal distance.

But a lens may magnify an image as well as an object. Thus (Fig. 6) M is a lens of short focus, A B an object just outside its principal focus. An image of A B is formed at *b a*. This is a magnified but inverted image. If now another lens, N, be placed so that the image *b a* is within its principal focus, the image *b a* is magnified still more, and appears at *b' a'* in the manner already described. The object, therefore, is magnified twice, and this is the principle of the compound microscope. The lens M, being near the object, is called the objective, N, being near the eye, is the eye-piece.

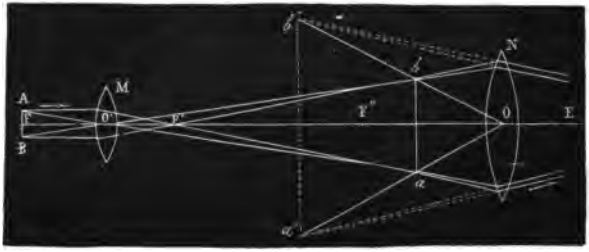

FIG. 6.

In the compound microscope, objective and eye-piece are each built up of several lenses, both to increase the magnifying power and to compensate for certain grave errors which a compound microscope of such simple construction as this always gives rise to.

It should be especially noted that the object must be distant from the objective rather further than its principal focal distance, that eye-piece and objective must be on the same axis, and such a distance apart that the image formed by the objective comes within the principal focus of the eye-piece, and that the eye must be placed near the eye-piece, in order to receive the divergent rays issuing from it.

The maker of the microscope places eye-piece and objective on the same axis, or should do so, and at the right distance apart, and provides a movable arrangement by which the objective may be brought to the proper distance from the object. The eye-piece merely magnifies an image already formed by the objective. Any defect in the latter is therefore magnified by the former according to its magnifying power, and movements are magnified in the same way. It is of prime importance, therefore, to have good objectives as well as good eye-pieces. Indeed, good glasses and a steady stand are the first things to look for in a microscope.

It has already been pointed out that we see an object by the light which comes from it. When, therefore, an object is magnified, the light coming from it is necessarily decreased in intensity. Thus, supposing that an object whose surface is one square millimetre is magnified so that its image occupies a space of one thousand square millimetres; since the image is formed by light from the object, this amount of light is now spread over a surface one thousand times as great as before, and, therefore, the light proceeding from every square millimetre of the image is only one thousandth of that arising from the object. Thus, it is impossible to greatly magnify a small object illuminated only in the ordinary manner, for the image might be so faint as to be scarcely able to excite the eye. Special means for concentrating light upon the object to be examined are therefore necessary for every compound microscope.

A transparent object which absorbs very little light requires much less illumination than a more opaque object. Too bright a light is also bad for the eyes. It is a safe rule to control the degree of illumination of the object in such a manner that the image shall not be much brighter than objects outside the microscope.

To control the amount of light falling on the object, as well as to cut off the rays which would otherwise pass through the margin of the lens, and distort the image, diaphragms are provided. Two forms are in general use. One consists of a metal plate with different sized holes in it. Any one of these can be brought in action by turning the plate around. The other is an arrangement by which a central hole can be increased or diminished so as to let in or shut out light by the movement of a lever. The latter, called the iris diaphragm, is by far the better.

Summing up what has been said, the requirements of a good microscope are—

(*a*) A firm and steady stand to carry the magnifying, illuminating, and accessory apparatus.

(*b*) Objectives and eye-pieces of different magnifying power. These must be of good quality, and must stand the following tests. The field must be flat, that is, when an object is in focus in any part of the field it must remain in focus if removed to any other part of the field. They must be achromatic, that is, there must be no coloured fringes along the edge of an image. The image formed by them must be clear and distinct in all its parts, not hazy and indistinct. Without good glasses, the best stands and accessory apparatus are useless.

(*c*) A stage to support the object to be examined.

(*d*) An efficient illuminating apparatus. This may consist of a mirror alone, which should have one plane and one concave side. The addition of a condensing apparatus to receive the light reflected from the mirror, and condense it on the object, will, however, greatly increase the efficiency of the instrument.

(*e*) An arrangement for raising or lowering the tube carrying the glasses so as to bring the object into focus. In the cheaper microscopes this is accomplished by sliding the tube inside a wide collar. For a slight additional expense, it is fitted with a rack and pinion for coarse adjustment to focus, and this is far preferable to the sliding arrangement. A fine adjustment is always provided. The coarse adjustment should work without backlash, that is to say, the smallest turn of the pinion should move the tube. The fine adjustment must work slowly and steadily.

(*f*) A good diaphragm, preferably of the iris form.

The best way of showing how these requirements are met is to describe a microscope which is suitable for the work of the student. There are many such in the market, both by

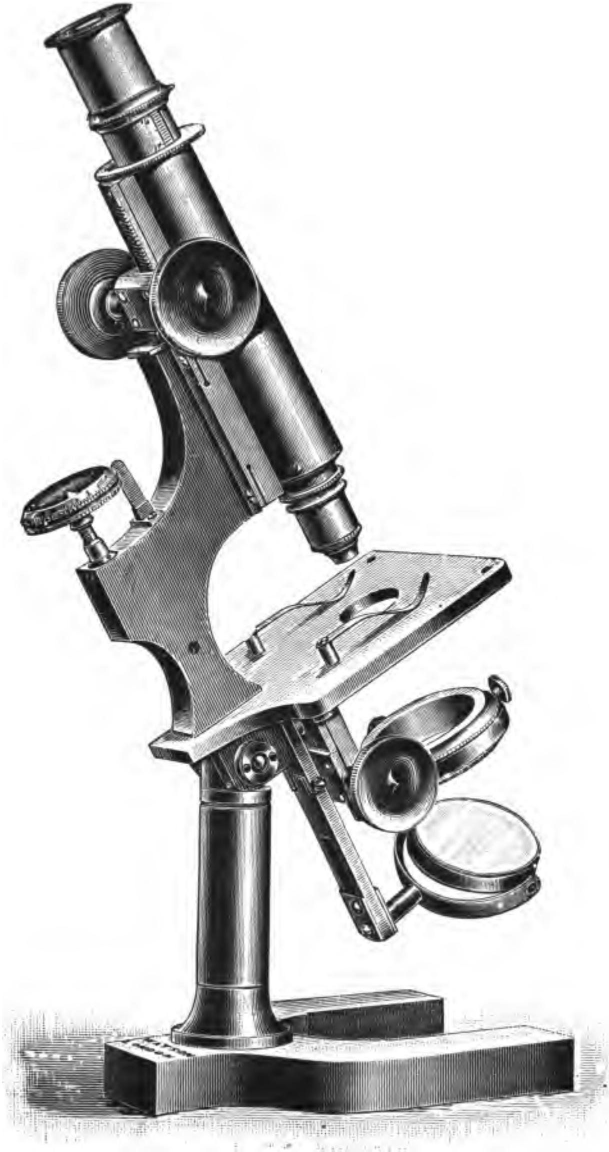

FIG. 7.

English and by foreign makers. The instrument figured on page 12 is one of the best of this class.[1]

The foot is of the continental horseshoe form, one which gives great stability whilst occupying a comparatively small space. From this rises a stout pillar which carries the rest of the instrument jointed upon it. The student will, as a rule, work with his microscope in the vertical position, and he must do so when he is using reagents on the stage; but it is a convenience to be able to incline it. The mirror is plane on one side, concave on the other, and is so mounted on a swinging arm that it can be placed in almost any position within the length of the arm. This allows of the most oblique illumination of the object being used.

Above the mirror is a sub-stage for carrying the condenser and diaphragm. It is possible to do good work, as good work has been done, without a condenser. If the student commences without it, he should look forward to possessing one some day, and his instrument should be provided with a sub-stage to carry it. The sub-stage is raised or lowered by a rack-and-pinion movement, and is mounted on a pivot, so that it can at any time be turned aside when direct light from the mirror is desired.

Above the sub-stage is the stage for carrying the object to be examined. This is provided with clips for holding the glass slip on which the object is mounted. More expensive microscopes frequently have elaborate mechanical contrivances for moving about the slip so as to bring different portions of it into the line of sight. This is, however, not necessary for the student who can use his fingers for this purpose.

The tube is of the continental length, about six inches, but is fitted with a draw-tube divided into centimetres, which, when it is out to its full extent, makes the length of the body about ten inches. The continental length is most desirable, because the instrument is not so long as to cause difficulty in using it in the vertical position.

It is moved by a spiral rack and pinion, which effectually prevents jerky movements. The fine adjustment, which is made by turning the milled-head screw at the back, is probably the best in existence. One revolution of the screw raises or lowers the body $\frac{1}{300}$ of an inch, and it is sensitive to the $\frac{1}{100}$ part of a revolution. The movement is very slow, steady, and smooth.

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[1] This is the Edinburgh Student's Microscope, Stand C, made and sold by Messrs. Watson and Sons, 313, High Holborn, London.

The lenses supplied with such an instrument are usually the B and C eye-pieces of English makers, and the one-inch and one-sixth of an inch objectives, the latter in either case the higher power. When the draw-tube of this instrument is closed, the one-inch objective with the B eye-piece gives a magnifying power of forty-three diameters, the one-sixth objective with the C eye-piece, under the same conditions, magnifies 336 diameters. With the draw-tube out, the magnification is greater, but with most microscopes what is gained in size in this way is lost in clear definition.

For the measurement of objects, an eye-piece micrometer must be obtained. This is a circular piece of glass with divisions engraved upon it, which drops into the eye-piece in such a position that its scale is projected upon the image, and can thus be directly compared with it. First, however, the value of its divisions with any combination of objective and eye-piece and length of tube, must be determined by comparing it with the scale engraved on a stage micrometer divided preferably in tenths and hundredths of a millimetre. Suppose, now, that with any combination of glasses, four divisions of the eye-piece micrometer coincide with one division of the image of the stage micrometer, then, under like conditions, four divisions of the eye-piece micrometer will always cover the image of an object which is actually $\frac{1}{100}$ of a millimetre in length, and consequently one division of the eye-piece micrometer will coincide with the image of an object $\frac{1}{400}$ of a millimetre in length. The value of the eye-piece scale is determined once for all for the combination, and objects can ever after be measured by it. The unit of microscopic measurement is $\frac{1}{1000}$ of a millimetre, and is usually written μ. It is equal to $\frac{1}{25000}$ of an inch nearly, and is called a micromillimetre.

Although the student should diligently practise rapid freehand outline sketching of objects under the microscope until he has acquired considerable power, an apparatus for drawing to scale is almost a necessity. There are many forms of camera lucida used for this purpose. All of them project the image on a piece of paper, at the same time allowing the point of a pencil to be seen whilst tracing over the image. Of such apparatus, Abbe's is perhaps the best. Beale's neutral-tinted reflector is cheap, and is fairly satisfactory to the student.

A very desirable piece of apparatus is a nose-piece for rapidly changing the objectives. Provided with a nose-piece, the lower-power objective can be used as a finder, then the higher power can be turned into the line of sight, and quickly racked down into focus.

To acquire facility in using the microscope, two or three prepared slides of transparent objects should be used. Place the instrument where the mirror is exposed to diffuse sunlight, or at night to a lamp giving a steady bright light, and provided with a shade such that although its direct light may reach the mirror, it is cut off from the eyes. Turn on the low power, look down the tube, and move the mirror until the field appears bright. Then place the slide on the stage so that the object is as nearly as possible over the centre of the hole in the stage, and rack the tube up and down until the object comes into view. Complete the focussing with the fine adjustment. If any portion of the object requires examination with a higher power, move it into the centre of the field, turn on the higher power, and rack down the objective into focus.

The greatest care must be taken that the objectives are not racked down to touch the slide. If this is done, there is risk of ruining slide or objective, or both. At the first time of using, close notice should be taken of the working distances of the objectives, so that mistakes may not be made in future.

With the object in focus, move the fine adjustment to get views of both surfaces of the object, and optical sections, as they are called, of its interior. Try the effect of altering the direction of the light, of racking the condenser up and down, of increasing and diminishing the amount of light which reaches the object through the diaphragm, and of increasing the length of the tube. Only by such experiments can the student learn the possible performance of his microscope. He should train himself to use either eye indifferently, and to keep both eyes open whilst using the microscope.

Objects to be examined by the compound microscope must necessarily be very small, and, if they are to be viewed by transmitted light, very thin. All bodies are transparent when in sufficiently thin plates.

The small objects must be mounted on slips of glass, in almost all cases in a drop of liquid, which will vary with the object, and covered with thin plates of glass called cover-glasses. Glass slips with smooth edges, and round or square cover-glasses, are sold by all dealers. No. 2 square cover-glasses, three-quarters of an inch square, will be best for the student. To mount the microscopic plant or animal in its living state, a drop of water should be placed as nearly as possible in the middle of the slip, the object placed in it, and the cover-glass let down obliquely upon it. Any surplus water can be taken up by applying a piece of blotting-paper to the edge of the cover-glass. Actively motile organisms should be so mounted that the cover-glass can not press upon them. This may be accomplished by using an excavated slip, that is a glass slip which has had a cavity ground out in it, or by placing something between the slip and cover-glass to take the pressure. A piece of glass-fibre, weed, cotton-wool, or anything of that kind, will serve.

An examination into the minute structure of the tissues of the larger animals and plants by means of the compound microscope necessitates preliminary preparations of the tissues. The tissue elements, which are almost always microscopic, may be isolated by mechanical or by chemical means. Or very thin sections of the tissues may be cut. In most cases both these methods of obtaining portions small and thin enough to examine will be used. The former will give good ideas of the forms and sizes of the elements, but no knowledge of how they are combined; the latter will show the combination of elements perfectly, but is not the best for the study of the individual elements.

32 Both for isolating the elements, and for cutting sections, fresh material is often suitable. Frequently, however, the tissue must undergo a special preparation to bring it to the right consistency for cutting, or to fill up its interspaces with some supporting material which will prevent it "giving" before the razor in cutting, or, if the contents of the tissue elements are to be studied in their natural position, to "fix" these contents. In addition it is often necessary to stain the preparation, either to render the whole more distinct, or, by using a stain which will colour one part and not another, to differentiate between elements, or between certain portions of their contents.

The isolation of elements may sometimes be effected by teasing out a portion of the tissue with fine needles. These should be set in wooden handles with about half an inch of the needles projecting. Then a small portion of the tissue should be placed on the slip in a drop of liquid, torn to pieces under the dissecting microscope, and then covered. The elements of the woody tissues of plants can be isolated by Schulze's Macerating Fluid of strong nitric acid and chlorate of potash. To use this, place a crystal of chlorate of potash in a test-tube, add a little strong nitric acid, and then drop in the pieces of tissue. The maceration will be complete in a few minutes if it be heated. It is better, however, not to heat, but to allow the fluid to act for at least a day at the ordinary temperature. For animal tissues, Muller's Fluid, prepared by dissolving 2·5 grams of potassium bichromate and 1 gram of sodium sulphate in 100 c.c. of distilled water, may be used. After the use of either, the elements may be separated by slight teasing.

Sections should be cut in at least two, and generally three, planes perpendicular to each other, and corresponding to the plan, front, and side elevations of the working drawings of the mechanic. If a preliminary teasing has shown that the elements of the tissue are prismatic, a transverse and one longitudinal section will as a rule be sufficient. From the stem of a plant, for example, transverse, radial, and tangential sections are required, and in not a few cases oblique sections are valuable. No general rule can be laid down. As he proceeds in his examination, the student will feel what sections are necessary in order that he may see through a structure from every point of view.

Soft tissues must be hardened for cutting. In all cases the tissue to be hardened should be in small pieces, in order that the hardening liquid may readily penetrate it, and the quantity of the reagent must be large or frequently changed.

Alcohol is the most general hardening reagent. Methylated spirit, which is about 90 per cent. alcohol, is commonly used. Absolute alcohol must be used for some purposes. When alcohol renders vegetable structures too brittle, they can be brought to the right consistency for cutting by steeping them in glycerine for some hours.

Chromic acid, in from a quarter to a half per cent. solution, is perhaps more used for animal tissues than for plants. After the acid has thoroughly permeated the tissue, the latter must be thoroughly washed in water, placed in weak, and finally transferred to strong alcohol, in which it may be kept. It is essential that all the chromic acid should be washed out of it. The weak alcohol must be changed as long as it is coloured by the tissue.

Picric acid in saturated solution is valuable for hardening and for fixing the contents of cells. It must, however, be completely washed out of the tissue before placing in alcohol or staining.

It must not be forgotten that alcohol dissolves out colouring matters, resins, and other substances from plant-cells, and that it precipitates many substances from their solutions in the cell-sap.

The first requisite for cutting good sections is a sharp razor. The razor whose edge will not bear examination under an inch objective is not fit for use. It is impossible for the student to take too much care of his razors. They should never be left about open; after use, they should be carefully washed, wiped, and polished, and preliminary sections, to obtain a good surface for cutting, should not be taken by the razor intended for cutting sections for examination.

For the actual cutting, an arrangement is required for holding the substance firmly in the right position, and for supporting the blade of the razor whilst it is being drawn through it. After each cut, the substance must be advanced the thickness of a section, in order to get the next cut.

The substance may be held between the thumb and forefinger of the left hand, and the blade of the razor may be rested on the fore finger while the right hand draws it through the tissue. This method, however, gives but indifferent results at the best; it is quite unsuited for cutting series of sections, and although some get fairly dexterous at it, others spend an amount of time endeavouring to acquire dexterity which may be better employed. A simple microtome suitable for doing all the work described in this book is a cheap necessity; small hand microtomes, consisting of a tube for holding the object, a screw for raising and depressing it, and a cutting-plate on which to rest the razor blade, may be obtained at as low a price as five shillings.

The more expensive freezing and other microtomes, which are elaborate machines, are not necessary for the student. He should, however, aim at possessing a microtome which will satisfy all or most of the following conditions. It should be of stout enough construction to be quite rigid; it should have a clamp, so that it may be fastened to the side of a table; the cutting-plate should be of polished glass; the milled head of the screw which raises the object should be provided with some sort of index, so that the object may be raised by definite amounts, and successive sections be of the same thickness; the plug in the tube should not revolve with the screw; and a clamp should be placed in the tube by which the imbedding or other substance which holds the object may be fixed. Messrs. Watson and Sons make and sell for fifteen shillings a Cathcart microtome which satisfies all these conditions except that of an index. This microtome has the advantage that at any time a freezing apparatus may be added to it for a few shillings.

The object to be cut must be held in the right position in the microtome clamp. Generally the student will place it between pieces of elder pith and fix the pith in the clamp. If the object is long enough and rigid enough, it may be placed between the pith with about $\frac{1}{12}$ of an inch projecting, and only the projecting portion cut through. This will be the method with transverse sections of plant stems, for example. In some cases longitudinal sections can be cut in this way if the piece of tissue is first trimmed to the right form and size. When transverse sections of smaller and less rigid objects are to be cut, as well as in the case of longitudinal sections of most objects of small diameter, the object should be held entirely between the pieces of pith, and pith and object cut together. In this case, the top of the pith should be trimmed off so that as little as possible of it should come into the section.

In the case of alcohol plant material, where only the arrangement of the elements is to be studied, and not their contents, it usually does not matter much if the object is squeezed out of shape in the clamp. The sections recover their size and shape when placed in glycerine. The student, however, must judge each case on its merits, and learn by experience what it is safe to subject to pressure in this way.

Very small and soft vegetable objects such as young flower buds should be imbedded in celloidin. The celloidin must be dissolved in a well-stoppered wide-mouthed bottle in a mixture of equal parts of absolute alcohol and ether. The object, which should have been some time in alcohol, is transferred to a mixture of alcohol and ether, and then to the solution of celloidin, in which it must remain until completely permeated by it. A cork to fit the microtome clamp should be taken, its smooth surface scored in order to allow the celloidin to hold, and a layer of celloidin spread on it and allowed to set, in order to prevent air-bubbles from subsequently rising. Then the object, in its drop of celloidin, must be quickly transferred to the top of the cork, and arranged in position. If the celloidin sets before this is accomplished, a drop or two of ether must be placed on it. When the celloidin is set hard enough to hold the object, the sections may be cut. Being transparent, there is no necessity to dissolve out the imbedding agent from the sections before mounting.

Useful sections of many hardened animal tissues may be cut from the portion projecting from between two pieces of pith. Most animal tissues, however, require to be imbedded. The agent universally employed is paraffin, which can be obtained of the right consistency from the dealers. The following directions, from Lloyd Morgan's "Animal Biology," will sufficiently describe the procedure :—

(*a*) Cut a small fragment fresh from the organ to be examined.

(*b*) Place in (i) osmic acid, 1 per cent., for two or three minutes; or (ii) in chromic acid, 5 per cent., for twenty-four hours; or (iii) in Kleinenberg's picric acid for eighteen hours. The object will thus be hardened.

(*c*) Place in 50 per cent. alcohol for twenty-four hours, then 70 per cent. alcohol for twelve hours, 80 per cent. alcohol for six hours, and transfer to 90 per cent. alcohol. This removes the water.

(*a*) Place in staining fluid until sufficiently deeply stained. Hæmatoxylin, picro-carmine, eosin, and borax-carmine are the most useful reagents. If borax-carmine be used, transfer it to 70 per cent. alcohol, to which a few drops of nitric or hydrochloric acid have been added.

(*e*) After staining, place for an hour in 80 per cent. alcohol, and then in absolute alcohol for another hour.

(*f*) Place in turpentine for an hour, and transfer to paraffin, which should be kept just melted. Leave for six or eight hours, and then cast in a mould. The small oblong vessels in which moist water colours are sold answer well. Arrange the specimen with warm needles.

(*g*) Pare away the paraffin so as to bring the object into view. Cut the thinnest sections you can with a sharp razor.

(*h*) Dissolve the paraffin in turpentine.

(*j*) The section may now be examined or mounted permanently in Canada balsam.

In the actual cutting, the blade of the razor must be laid flat on the plate of the microtome, and pushed from the operator across and not directly through the object. The razor may be dry in cutting animal tissues prepared as above, but in cutting vegetable tissues it must be kept flooded with water if the tissue is fresh, with the preserving fluid if the material has been preserved.

The sections may be removed from the razor-blade on the point of a fine brush, some may be safely removed on the point of a needle, or they may be flooded off into a watch-glass by liquid discharged from a pipette.

Such sections as are to be examined without further treatment may be placed at once in a drop of the mounting liquid on a glass slip and covered. Animal sections are, as a rule, stained before cutting, vegetable tissues after. Where the form and arrangement of the elements in a vegetable tissue are alone to be studied, the contents of the cells will be dissolved out by immersing the sections in a few drops of eau de Javelle for a few minutes, then in very weak acetic acid, and finally washing well with water. They can then be stained if necessary.

Sufficient directions for staining are given throughout the book, but one general rule must be borne in mind. Dilute solutions of staining fluids are slower in their action than stronger solutions, but they produce by far the best results.

If the student wishes to preserve his preparations for the microscope, his mounting media will, as a rule, be glycerine, glycerine jelly, or Canada balsam.

Most of his vegetable preparations will be mounted in glycerine or glycerine jelly. The object to be mounted in glycerine should first be placed in a mixture of alcohol and glycerine, and kept there until the fluid has concentrated by the evaporation of the alcohol. Then a drop of glycerine should be placed on the glass slip, the object placed in it, and a cover-glass gently lowered obliquely upon it from the point of a needle. Any surplus glycerine must be taken up by a piece of blotting-paper from the edge of the cover-glass. After some practice, the student will find he can judge of the amount of glycerine required, so as to avoid excess. A ring of gold size, repeated several times as it dries, must be laid on with a brush to close in the preparation, but the gold size will not adhere to the glass where it is smeared with glycerine.

If glycerine jelly is used, a small piece is placed in the middle of the slide, and rendered fluid by gently warming. The object should then be placed in the drop, and covered. The jelly hardens around the rim of the cover-glass, which therefore requires no closing in.

Farrant's medium is fluid at the ordinary temperature, and may be used instead of glycerine jelly. It also hardens at the edge of the cover-glass.

Animal sections cut in paraffin are mounted in Canada balsam. Howes, in his "Atlas of Biology," gives the following directions for doing this :—

"Transfer the sections as cut, together with the imbedding material in which they lie, to a slide, the surface of which has been previously painted over with a heated solution of white shellac in kreasote. Submit the whole to the temperature of the melting point of the paraffin until the kreasote has evaporated, when the sections will become firmly adherent to the shellac. The slide must next be immersed in turpentine, which will dissolve up the remaining imbedding material, thus leaving the sections fixed in place and ready to be covered with Canada balsam.

"For final mounting, well-powdered Canada balsam, first thoroughly dried, must be dissolved in benzole or chloroform, to the consistency of glycerine. A drop of the balsam thus prepared should be deposited upon the slide by means of a glass rod, and allowed to diffuse itself among the sections; the cover-glass should then be let down gently and obliquely upon the objects, its under surface first having been smeared with Canada balsam."

The following is a short list of reagents, etc., which the student should supply himself with. All may be obtained from dealers, although the student may himself prepare some. The number following each refers to a page on which its use is described or directed :—

Micro-chemical Reagents.

Iodine (29) solutions in alcohol and in potassium iodide.

Iodine (47) solution in zinc chloride (Schulze's solution). This is made by saturating pure hydrochloric acid with zinc, and then adding a very strong solution of potassium-iodide-iodine.

Fuchsin (122) solution in small amount of alcohol diluted with water. The alcoholic solution mixed with glycerine stains slowly, but produces excellent results.

Ether and chloroform (185), used as solvents for fats.

Aniline chloride (121) in water acidulated with hydrochloric acid.

Ammonia and nitric acid, used as a test for proteids, which they stain bright yellow.

Copper sulphate (34), used with caustic soda and tartaric acid in Fehling's liquid as a test for sugar.

Corallin (160) in sodium carbonate solution.

Olive oil (185), for mounting aleurone grains.

Osmic acid (81), for staining fats and oils.

Potash in water and in alcohol, for rendering sections transparent.

Sulphuric acid (121), concentrated.

Sulphuric acid (29), dilute.

Chlorate of potash (124).

Sodium chloride (319) (normal salt solution, ·75 per cent.).

Muller's fluid (17), for hardening and macerating animal tissues.

Pfeffer's fluid (185), for rendering permanent aleurone grains.

Preserving Fluids.

Methylated spirit (3), for general material.

Absolute alcohol (18), for material in which the cell-contents are to be studied.

Hardening Fluids.

Alcohol, methylated (18).

Chromic acid (18).

Picric acid (107).
Osmic acid (21).

Imbedding Substances.

Paraffin (21).
Celloidin (20).

Clearing Reagents.

Eau de Javelle (Potassium hypochlorite) (122).
Potash in water or in alcohol.

Staining Fluids.

Gentian violet (61).
Methyl green, for nuclear staining.
Hæmatoxylin (107), solution in ammonia, for nuclear staining.
Fuchsin (122).
Acetic aniline green.
Hamann's carmine (221).
Borax carmine (21).

The following is a short list of books in English, which the student may profitably consult in order to supplement and extend the information contained in the following chapters. It does not pretend to be comprehensive :—

The Microscope.

Beale, "How to work with the Microscope."
Carpenter, "The Microscope."

Practical Exercises in Biology.

Strasburger and Hillhouse, "Practical Botany."
Bower, "Practical Botany."
Marshall and Hurst, "Practical Zoology."
Foster and Langley, "Practical Physiology."
Howes, "Atlas of Practical Elementary Biology."

Botany.

De Bary, "Comparative Anatomy of the Phanerogams and Ferns."
De Bary, "Comparative Morphology and Biology of the Fungi," etc.
Gœbel, "Outlines of Classification and Special Morphology."
Bennett and Murray, "Cryptogamic Botany."

Goodale, "Physiological Botany."
Sachs, "Lectures on Plant Physiology."
Vines, "Physiology of Plants."

Zoology.

Gegenbaur, "Elements of Comparative Anatomy."
Huxley, "The Anatomy of Invertebrated Animals."
Huxley, "The Anatomy of Vertebrated Animals."
Ecker, "Anatomy of the Frog."
Marshall, "The Frog."
Huxley, "The Crayfish."
Lloyd Morgan, "Animal Biology."
Lankester and others, Zoological Articles contributed to the "Encyclopædia Britannica."

CHAPTER I.

YEAST (SACCHAROMYCES—SPROUTING FUNGI).

1. TAKE some brewer's yeast (*Saccharomyces cerevisiæ*), widely used for exciting fermentation in the wort of beer, and notice its semi-fluid character and light-brown colour. A low power of the microscope is sufficient to show that it consists of small particles suspended in a thin liquid. Allow it to stand for a time, and it separates itself into a deposit on the sides and bottom of the vessel, and a clear supernatant liquid. The deposit is the **Yeast**; the liquid is mainly water.

2. Dry some yeast by exposing it to the atmosphere or to a gentle heat. A powdery mass of yeast is obtained.

3. Heat some of the dry yeast in a porcelain or other crucible over a spirit-lamp or Bunsen gas-burner. It first loses a quantity of water which is not expelled from it at the ordinary temperature of the air, next blackens, and finally burns, leaving a small quantity of white ash. The blackening of the yeast indicates the presence of carbon. Appropriate chemical experiments show also that compounds of carbon, oxygen, hydrogen, and nitrogen were given off during the burning, and that the ash contains the elements sulphur, phosphorus, potassium, magnesium, calcium, and possibly some others in small quantities.

4. Place a small drop of yeast on a slide, cover with a cover-glass, and examine under a high power of the microscope. A large number of spherical or slightly ovoidal colourless **Cells** are seen. Each consists of a very delicate outer limiting membrane, or **Cell-wall**: this is a closed bag containing a semi-fluid substance—the **Protoplasm.** In the protoplasm of many cells will be seen clearer round or oval spaces—the

Vacuoles—containing a watery **Cell-sap**, and scattered throughout the protoplasm of some cells will be seen a crowd of minute refractive particles of fat, which have the effect of making the protoplasm appear granular. The protoplasm of other cells will be clear and homogeneous.

5. Measure some of the cells. They will be found to vary in size from 8μ to 14μ (from ·00031 to ·00055 inch).

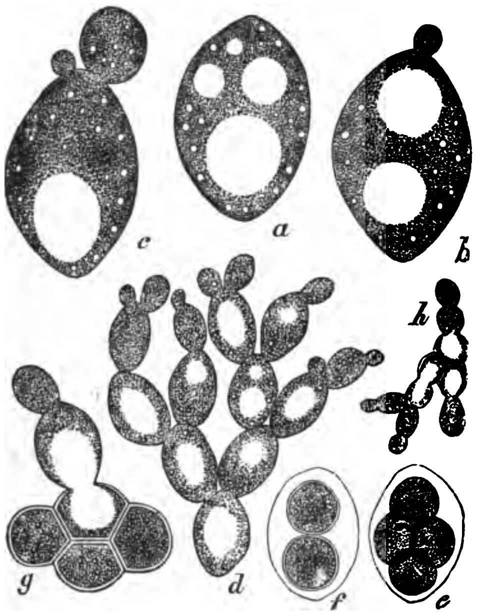

FIG. 8.—*Saccharomyces cerevisiæ*. *a*, single cell; *b*, *c*, stages of sprouting; *d*, growth-form; *e*, *f*, cells with ascospores; *g*, an ascospore sprouting. (*a–d*, Bennett and Murray; *e–h*, Reess.)

6. Press sharply on the cover-glass. Some of the cells are burst, and the protoplasm, which after a short time becomes diffused throughout the water, exudes. Many of the highly refractive granules of the protoplasm, however, retain their individuality for a considerable time. Note the empty cells (membranes).

7. Spread a little yeast thinly on a slide with a camel's hair brush, and allow it to dry thoroughly. It adheres so firmly to the glass that it may be treated with reagents, washed, and mounted in this position, without being detached. The outer layer of the cell-wall is **Mucilaginous**, and the cells adhere to the slide and to each other by means of this mucilage.

8. Add a drop of solution of iodine in potassium iodide to

the dry layer of yeast on a slide, and allow it to remain for a few minutes. Wash lightly, mount in water, and examine under the microscope. The cell-wall is stained very slightly, the protoplasm takes on a brown colour, and the whole structure of the cell is rendered more evident. **Starch** is not present, or it would have taken on a blue colour with the iodine. Note that since the cell is a closed sac the iodine must have passed through the cell-wall in order to stain its contents.

9. Treat a similar dry layer of yeast with iodine solution and then with 70 per cent. sulphuric acid; wash lightly, and mount in water. All the cells are stained, some deeply. The cell-wall takes on a yellowish-brown to brown coloration.

Most cell-walls of the higher plants are stained blue when treated successively with solution of iodine and sulphuric acid, and this reaction is characteristic of their substance, **Cellulose**, which has the composition $(C_6H_{10}O_5)_n$, and is one of the class of compounds called **Carbohydrates.** The cell-wall of yeast is also composed of cellulose, but, in common with the cell-walls of most other fungi, it is either in a different physical condition or it has an admixture of other substances which prevent the reaction with these reagents. Probably the latter is the case. The material of these cell-walls has received the name **Fungus Cellulose.**

10. Treat a similar dry layer of yeast with 70 per cent. sulphuric acid, allow to act for five minutes, wash and mount in water. The contents of most of the cells will be disorganized, the cell-walls resist the acid.

11. Treat, as in 10, with sulphuric acid, wash, and stain with potassium iodide iodine. It will be seen that in those cells whose contents have not been disorganized the protoplasm, stained dark brown, has shrunk away from the cell-wall, allowing the latter to be seen with great ease.

The yellowish-brown colour which protoplasm assumes with iodine is characteristic of this substance.

12. Treat yeast on a slide with solution of potash or of potassium hypochlorite (*eau de Javelle*). The protoplasm is dissolved, and nothing is seen to be left but the empty cell-walls.

Protoplasm as we see it in living cells is a fluid whose viscosity varies in different plants and animals within fairly wide limits. It is a very complex but organized association of many chemical compounds, of which the following are the chief:—

(*a*) Albuminous compounds or proteids, which are of the same nature as the casein of milk, the fibrin of blood, and the albumin of egg. Three classes of proteids can be recognized in protoplasm—

(i.) **Peptones**, which are soluble in water.

(ii.) **Globulins**, not soluble in water, but soluble in dilute potash solution.

(iii.) **Plastin**, which is soluble in strong potash solution. All proteids are composed of the elements carbon, hydrogen, oxygen, nitrogen, and sulphur, and in addition plastin contains phosphorus.

(*b*) Some mineral compounds, the elements of which form the ash constituents (*vide* s. 3).

(*c*) Water, which is an integral portion of the protoplasm.

The constituent compounds of the protoplasm are banded together in such a manner as to make, not a form whole, like a crystal, but a functional or organic whole; not a mere mixture of compounds, nor one single chemical compound, but an association of compounds, the link between which being broken, the substance is no longer protoplasm, but a mixture of its constituent compounds. It is the substance in which resides the life of the plant or animal, the seat of all vital activity. In a living cell constant chemical changes are going on in the protoplasm and by its agency, such chemical changes being the outward manifestation of its life. Nutritive compounds, organic and inorganic, are absorbed by it, passing through the cell-wall, where there is one, and penetrating the protoplasm, in the form of solutions in water. These compounds are decomposed by the aid of energy, also absorbed from without, and are finally incorporated into the substance of the protoplasm (**Constructive Metabolism**). Portions of it, or products of its metabolism, are constantly being separated from its mass to be absorbed again, or to form a stock of reserve nutritive material, or to provide substances whose function shall be partially or wholly mechanical. Thus the cell-wall is formed and added to by the formation of cellulose out of protoplasm. Lastly, by the absorption of oxygen some of its constituents are continually being converted into more highly oxidized compounds of simpler constitution (**Destructive Metabolism**), giving off heat in so doing, the compounds thus formed being excreted from the protoplasm (**Excretion**). So long as the protoplasm is alive destructive metabolism continues, even if it be not constructively metabolic, and after death the whole is resolved into carbon dioxide (CO_2), water (H_2O), nitrogen, or ammonia (NH_3), phosphates and sulphates of calcium, magnesium, and potassium, and under varying conditions into other compounds.

Since such is the composition of yeast, it is obvious that no increase in the weight of any quantity of it can take place unless a supply of these ash and other constituents are at the disposal of the plant. They cannot, however, be used, with the single exception of oxygen, in the form of elements, but must be presented as compounds. The list of compounds available for the purpose is not by any means a large one. The ash constituents and hydrogen must generally be presented in the form of inorganic compounds, the nitrogen may be in the same form, but the carbon must be contained in an organic compound.

The simplest mixture of nutritive substances in which yeast can be cultivated is the solution first used by Adolf Mayer in 1869. It is composed as follows:—

Distilled Water	H_2O	500 cub. centimetres
Sugar Candy	$C_{12}H_{22}O_{11}$	15 grams
Ammonium Nitrate	NH_4NO_3	1 gram
Dihydric Potassium Phosphate	KH_2PO_4	0·5 ,,
Tricalcium Phosphate	$Ca_3(PO_4)_2$	0·05 ,,
Magnesium Sulphate	$MgSO_4$	0·25 ,,

Unless special means be taken for expelling it, oxygen will also be held in solution in this mixture, and except in case of complete isolation it will also be freely absorbed from the atmosphere. It will thus be seen that Mayer's solution contains all the necessary elements for the construction of the protoplasm, cellulose, fat, and other compounds found in the yeast plant; and if yeast lives and increases in it, as it does, it must obtain its carbon from the sugar, its nitrogen from the ammonium nitrate, its potassium from the acid potassium phosphate, its calcium from the calcium phosphate, its sulphur and magnesium from the magnesium sulphate, and its phosphorus from one or both the phosphates. Its hydrogen it can obtain from water, and it can absorb some of the free oxygen in solution.

It has been shown that yeast burns, giving out energy in the form of heat in so doing. Yeast thus contains a store of potential energy, which, since energy cannot be created by the plant, must be obtained by it either from the compounds which serve it as food or from some other source. The only compound in Mayer's solution which is not fully oxidized is the sugar, and from the conditions under which yeast thrives the only other available source of energy for it is external heat. From one or both these sources the energy which is stored up in the yeast plant must be obtained.

Any organism which, like yeast, has the power of using the nitrogen of an inorganic compound such as ammonium nitrate in the construction of protoplasm is an undoubted plant.

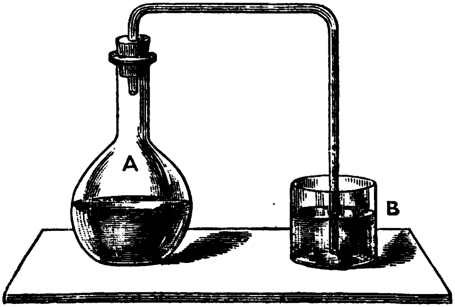

FIG. 9.—A, flask containing yeast in nutrient solution; B, vessel containing lime-water.

13. Prepare Mayer's solution, and place in two flasks, A and B.

A about half full. Fit the flask with a cork, through which passes a delivery tube bent twice at right angles, and let the end of the delivery tube dip into a vessel containing lime-water. Add a few drops of vigorous young yeast to the solution in the flask, and keep at a temperature of 25° to 30° C.

B, similar in all respects to A, but not sown with yeast.

In a few hours the liquid in A becomes turbid, froths up, and disengages bubbles of carbon dioxide gas (CO_2), which pass through the lime-water and render it milky. In B no turbidity is seen and no carbon dioxide is produced. Whilst fermentation is most active test the temperature in each flask. That of A will be higher than, that of B the same as, the temperature of the surrounding air. Thus heat is produced in fermentation. Allow the flasks to stand for some days until all fermentation has ceased, that is, until there is no further production of carbon dioxide. Note the beery smell of A, and the absence of any such smell in B. Also note the thick deposit on the sides and bottom of A. This will be found to be yeast similar to that added, but obviously in much larger quantity.

14. Place some of the liquid from A in a small retort, and distil over about one-fifth of it. Make this into a paste with quicklime and distil again.

Note especially at the commencement of the second distillation the streakiness on the sides of the neck of the retort. This is characteristic of **Alcohol**, and a very small proportion of it may be detected with certainty by this means. The distillate burns with an almost invisible flame. If three or four drops of a solution of potassium bichromate be added to some of it, then a few drops of sulphuric acid, and the mixture be heated, a green colour and a fruity odour are produced. This last reaction is also a certain indication of the presence of alcohol.

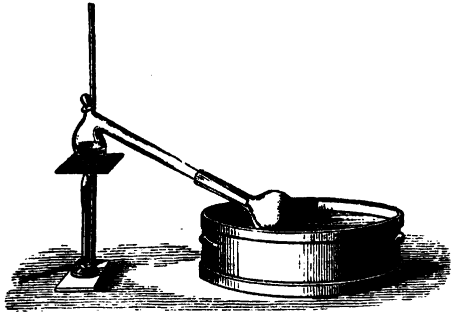

FIG. 10.—Apparatus for the distillation of alcohol from fermented sugar solution.

15. Test the contents of B in the same way. No alcohol is found.

16. Prepare an alkaline copper tartrate solution (*Fehling's solution*) by adding tartaric acid to copper sulphate solution until the precipitate first formed is redissolved, and then rendering it strongly alkaline with sodium hydrate. To this add some of the liquid from A, and boil. If fermentation has been complete there is no precipitate of red copper oxide, showing that the sugar has disappeared.

17. Test some of the liquid from B in the same way by boiling well with Fehling's solution, and abundance of sugar is shown to be present. If a single drop of sulphuric acid be added to the boiling liquid the precipitate appears in much less time.

Cane-sugar, $C_{12}H_{22}O_{11}$, reduces Fehling's solution only after long boiling; but if Mayer's solution when in active fermentation with yeast be tested for sugar, a copious precipitate of copper oxide is obtained on slightly warming. The explanation is that the first effect of the yeast on the cane-sugar is to convert it into **Glucose** or **Grape-sugar,** $C_6H_{12}O_6$, which reduces Fehling's solution very readily.

18. Sow Mayer's solution with yeast, and keep at or near the freezing point of water. There is no fermentation. For this, heat from without is necessary. The **Minimum Temperature,** below which fermentation does not take place, is 9° C.; the **Optimum Temperature,** at which it is most active, is between 25° C. and 35° C.; and the **Maximum Temperature,** above which there is no fermentation, is below 60° C.

We are now in a position to formulate the results of these experiments. In the first flask, A—

Heat has been absorbed from without.
The yeast has increased in quantity. And therefore
Energy has been stored up.
Heat has been given out.
Sugar has disappeared.
Alcohol and carbon dioxide have been produced.

In B there has been no change.

The inference is that in A these changes have been brought about by the yeast added to it.

The increase in the quantity of yeast signifies an increase in the quantity of proteids, cellulose, fat, and mineral constituents, and it is clear that these additional organic compounds must have been formed out of the compounds in the nutrient solution, in the midst of which the cells of the yeast plant live. Each nutrient compound continues to pass through the cell-wall and to reach the interior of the cell so long as its solution in the cell-sap is less concentrated than its solution outside, and since these nutrient compounds are being continuously decomposed in the cell, their passage inwards is also continuous. The movement of substances through a membrane from a more to a less saturated solution is known as **Osmosis.** In the cell, decomposition first takes place, and for this energy is necessary. A small part of this energy is absorbed from without in the form of heat. Recomposition then ensues and protoplasm is formed. Not more than one per cent. of the total sugar which disappears goes to form yeast; of the remainder about 95 per cent. becomes decomposed into carbon dioxide and alcohol, and the rest into glycerine, succinic acid, and carbon dioxide.

19. Make up Mayer's solution without ammonium nitrate, sow with a small quantity of actively growing yeast, and keep at the fermenting temperature. There will be no fermentation, or at most it will be barely perceptible. That there is any fermentation at all is owing to the fact that the yeast can feed for a time on its very large reserve of nitrogenous matter.

20. Make up Mayer's solution without sugar, sow with a small quantity of active yeast, and keep at the fermenting temperature. There is occasionally a small production of carbon dioxide and alcohol, but they are formed out of the elements of the yeast.

21. Make up Mayer's solution without phosphates. Such a medium also will allow of but the faintest indications of fermentation or of none at all.

The three preceding experiments show that fermentation is practically impossible unless the yeast plant is supplied with suitable carbonaceous, nitrogenous, and phosphorus compounds as food. The other constituent appears to be of less importance.

It has been explained that the constructive metabolism of the protoplasm converts the products of the decomposition of the nutritive compounds into protoplasm itself. This is essentially an association of chemical compounds the affinities of whose elements are not completely satisfied. The protoplasm thus formed is therefore not only a store of matter but of potential energy, since, by such a simple process as burning, energy in the form of heat can be obtained from it. This energy cannot be created by the plant. A very small portion is absorbed from without in the form of heat; much the larger portion is supplied to the plant in the form of potential energy of sugar. But heat also passes away from the yeast during the whole period of fermentation, and this is so much energy lost in addition to that stored up. This production of heat may be very large; it is directly proportional to the amounts of yeast produced and of sugar fermented. Naegeli calculated that in the fermentation of one kilogram of cane-sugar, or after its inversion of 1·0526 kilogram of grape-sugar, 146·6 thermal units appear. The heat set free has a double origin. A portion of it is produced in the destructive metabolism of the protoplasm, in which process carbon dioxide and water are formed (**Respiration**). Destructive metabolism is continuous whether the plant be fed or not. If yeast be placed in distilled water, after a time the protoplasmic contents of the cells have perceptibly diminished, so that an actual loss of substance has taken place. The other portion is produced in the decomposition of the sugar into carbon dioxide and alcohol. The amount of heat set free in this decomposition is about one-fifteenth of the total amount which would be produced by the complete combustion of the sugar.

The simultaneous disappearance of sugar and appearance of carbon dioxide and alcohol suggest an intimate connection between the two phenomena, and accurate investigation has demonstrated that by the living activity of the yeast the sugar has been directly decomposed into alcohol and carbon dioxide. This decomposition may be represented by the equation—

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{Alcohol.}}{2C_2H_6O} + \underset{\text{Carbon dioxide.}}{2CO_2}$$

According to this equation one gram of grape-sugar will on decomposition yield 0·49 gram of carbon dioxide and 0·51 gram of alcohol. And it has been found by direct experiment that when a sugared solution is fermented with yeast, the relationship between the amounts of sugar disappearing and of alcohol and carbon dioxide appearing is approximately the same as given by this equation.

22. Fill a flask with Mayer's solution, and boil it well in the flask for an hour, keeping the flask full by adding distilled water. When cool sow with actively growing yeast, and cork tightly with a good cork, having passed through it a tube bent at an angle of 60°. Let the free end of the tube dip in a vessel containing well-boiled distilled water, or preferably mercury, and keep the flask at the fermenting temperature.

The solution is boiled to expel air. Pasteur found, in dealing with a sugared solution of somewhat similar composition, that after boiling and cooling, three litres of the solution contained less than $\frac{1}{1000}$ gram of oxygen. Our flask then will certainly contain but an extremely small quantity of oxygen, and as arranged, it is cut off from all air except that in the tube. Under these circumstances it will be found that the fermentation is slow but decided. After several days it generally happens that there are still evidences of fermentation going on—small bubbles of gas are still rising to the top of the flask.

The fact is well established that yeast can increase in quantity and effect fermentation in the absence of oxygen, in that case getting its oxygen for destructive metabolism from the sugar. But sooner or later the cells die when deprived of free oxygen. Pasteur says, "To multiply in a fermentable medium out of the presence of oxygen gas, the cells of yeast ought to be extremely young, full of life and health, still under the influence of the vital activity which they owe to the free oxygen which has served to form them, and which perhaps they have stored up for a time. Older, they have great difficulty in reproducing themselves without air, and they age more and more. If they multiply at all it is under a form fantastic and monstrous. Older still, they remain absolutely inert in a medium deprived of oxygen." He classes this particular form of yeast as an **Aërobiotic** form, because free oxygen is a necessity for its life. There are, however, **Anaërobiotic** forms which thrive best without free oxygen, but they are few.

The amount of sugar which is decomposed into alcohol and carbon dioxide for a given weight of yeast formed is not a constant quantity, and depends on the amount of free oxygen at the command of the plant. Pasteur experimented with two solutions. One contained free oxygen, the other was deprived of, and kept isolated from, free oxygen. Both were sown with yeast and kept under similar conditions. The proportion between the yeast formed and sugar decomposed in the first solution was 1 : 76; in the second, 1 : 89. So that with free oxygen there was not so much sugar decomposed for the same amount of yeast formed, as there was without free oxygen, although in the first case the absolute amount of yeast formed was greater than in the second case.

It is to be especially borne in mind that the decomposition of sugar by the agency of yeast is of two kinds. About one per cent. is decomposed in the cell, and its elements taken up by the cell for its own nourishment. The rest is decomposed, also within the cell, but the products of the decomposition speedily pass out of it. This decomposition sets free heat, which the plant gets the benefit of. *Any organism which is capable of bringing about the decomposition of its substratum in the way that yeast brings about the decomposition of sugar into alcohol and carbon dioxide, is a ferment* (*Organized Ferment*). The character of the effect of a ferment on its substratum is constant. It is always the most nutritive compounds which are destroyed, and the products of the decomposition are always compounds which are less suitable for nutrition.

23. Add yeast to Mayer's solution in a flask, and then boil

for a short time. Immediately plug the neck of the flask with cotton wool or asbestos, in order that any air which enters on cooling may be filtered from germs, and keep under the proper conditions for fermentation.

The liquid does not ferment. A boiling temperature destroys the power of yeast to bring about fermentation by destroying the yeast itself. The fatal temperature for moist yeast is not nearly so high as this, being in fact about 60° C. Schützenberger states that dry yeast may be heated to nearly 100° C. without being killed. Caignard de la Tour found that dry yeast may be cooled to $-60°$ C. without losing its vitality, whereas when moist it is killed at $-5°$ C.

24. Sow yeast in Mayer's solution, and keep in a dark place at the fermenting temperature.

Fermentation goes on as well in the dark as in the light. Yeast is independent of light for its growth, whereas all green plants require it.

It has been already stated that the first effect of yeast on cane-sugar is to convert it into glucose or grape-sugar, and that cane-sugar reduces Fehling's solution only after long boiling.

25. Make a solution of cane-sugar in water, or take Mayer's solution, and add to it some water in which yeast has been washed. Test immediately with Fehling's solution, and a copious red precipitate is obtained on warming, showing that the cane-sugar has been quickly changed into grape-sugar by the action of the yeast-water. The change may be represented by the following equation :—

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + \underset{\text{Water.}}{H_2O} = \underset{\text{Grape-sugar.}}{2C_6H_{12}O_6}$$

The nature of this change must be noted. It involves the fixation of the elements of water and a subsequent decomposition under the influence of a substance which although not living is of vital origin, being, in fact, dissolved out from yeast-cells where it is formed. A change of this kind is one of **Hydrolysis**, and the influencing substance is a **Hydrolyst** (**Enzyme**, or **Unorganized Ferment**).

The income and expenditure of yeast with respect to both matter and energy may be thus tabulated—

INCOME.	EXPENDITURE.
Matter: Sugar, inorganic salts, water, free oxygen.	*Matter:* Loss of carbon dioxide and water in respiration. Excess of cell-formation.
Energy: Potential energy of sugar. Heat (kinetic energy).	*Energy:* Heat evolved. Work done in growth. Potential energy of organic compounds formed.

IN FERMENTATION.

Matter: Sugar.	*Matter:* Alcohol, carbon dioxide.
Energy: Potential energy of sugar.	*Energy:* Heat evolved. Potential energy of alcohol.

BALANCE IN FAVOUR OF THE YEAST.

Matter: Proteids, cellulose, fat, etc., of cells formed.
Energy: Potential energy of organic compounds formed.

Mayer's solution is not the only mixture of nutritive compounds in which yeast will thrive, nor is it the most suitable. An organic compound like ammonium tartrate, $(NH_4)_2C_4H_4O_6$, may replace ammonium nitrate with advantage, and a mixture in which this exchange is made is known as **Pasteur's Solution.** Ammonium compounds are good, and nitrates indifferent, sources of nitrogen for yeast. Free nitrogen cannot be assimilated. But no substance is so suitable to supply nitrogen to yeast as those nitrogenous compounds which occur so plentifully in such natural fluids as the wort of beer, the juice of sugary fruits, and the water of yeast. They contain peptones (soluble proteids), which are capable of diffusing through the cell-wall. Living in such a medium, the plant is obtaining both its carbon and its nitrogen from organic compounds which are also capable of nourishing animals. The peptones formed in animal digestion are also eminently suited for the nourishment of yeast.

Sugar is its best source of carbon, although almost any carbonaceous compound which is soluble and not too poisonous is available for the purpose so long as the plant has plenty of free oxygen. It cannot, however, obtain its carbon from carbon dioxide. If well supplied with oxygen, yeast-cells multiply in yeast-water, getting their carbon in that case from nitrogenous carbon compounds. There cannot, of course, be any alcoholic fermentation in such a liquid, and, speaking generally, the development of yeast is very difficult in a non-sugared medium.

The best nutritive solution is one containing peptones and sugar with the mineral constituents of the ashes of yeast.

Reproduction.

26. Examine some actively growing yeast under the microscope, and note that many of the cells are attached together in growth-forms, which are branched strings of cells. The cells separate from each other on a slight disturbance. If the yeast be cultivated in nutrient solution on the slide, these growth-forms will be larger and more beautiful because less disturbed. The manner of their formation is this. An actively growing cell develops a slight protuberance at a point—which may be at the extremity of the cell or may not—or at more than one point. This enlarges, is filled with protoplasm from the mother-cell, and becomes constricted at the point of origin. Eventually a partition-wall is formed there, but unless a slight force is used the cells remain attached. Generally before the **Sprout-cell** has attained its full size it also commences sprouting, and so on. The branched sprout-chain constitutes a very simple **Mycelium.** This method of reproduction is a vegetative one; each sprout-cell is similar in all respects to the cell from which it sprouted, and every cell is capable of sprouting.

The necessary conditions for the growth and reproduction of a yeast-cell are a supply of water, of food, of oxygen or fermentable matter, and a proper temperature. The protoplasm of the young cell exerts a pressure on the cell-wall, thus stretching it, and this increase in size is rendered permanent by the deposition of cellulose on the cell-wall. This continues until the cell has reached its full size. Meanwhile the protoplasm has also been increasing in quantity, but frequently not quickly enough to keep the cell quite full, so that vacuoles appear full of cell-sap.

There is another method of reproduction. When well-fed yeast is suddenly deprived of all nourishment, especially sugar, but kept constantly moist, supplied with oxygen, and at a suitable temperature, it forms spores.

27. Prepare a small block of plaster, and smear the top of it with a thin layer of actively growing yeast which has been washed in distilled water. Stand it in a vessel and pour in distilled water until it reaches to within half an inch or less of the top. Cover with glass to exclude dust and atmospheric germs. Examine from the second day. The contents of the older cells disappear, but at from two to four points in the younger cells small masses of denser and more refractive protoplasm collect, slowly increase in size, and eventually become coated with membranes. These are **Ascospores** formed by the process of **Partial Division** (free cell-formation) from the protoplasm of the mother-cell, which is an **Ascus.** The wall of the ascus disappears and in a nutrient solution the ascospores sprout, giving rise to vegetative cells.

Any plant which, not being green, is dependent on organic compounds as its source of carbon, and whose structure and modes of reproduction are as have been described above, is a **Fungus.**

Saccharomyces cerevisiæ is not the only species of Saccharomyces which can cause alcoholic fermentation in sugary nutritive solutions.

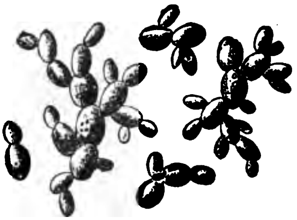

FIG. 11.—*Saccharomyces ellipsoideus* budding. (Schützenberger.)

S. ellipsoideus is the ordinary yeast of wine. The cells are ellipsoidal, and have a length of 6μ and a breadth of from 4μ to 5μ. The mode of forming ascospores is as has been described for *S. cerevisiæ.*

S. Pastorianus is also a ferment of wine. Its cells are oval in outline and small, or else elongated up to 20μ in length, one end of the elongated cell being usually swollen.

This difference of form is a consequence of differences of the conditions under which it lives. Fig. 12 represents *S. Pastorianus* in its ordinary form, and Fig. 13 shows it after successive cultivations in nutritive fluids. Ordinarily the ferment appears spontaneously in wines shortly before the termination of fermentation. Ascospores have been observed in this species.

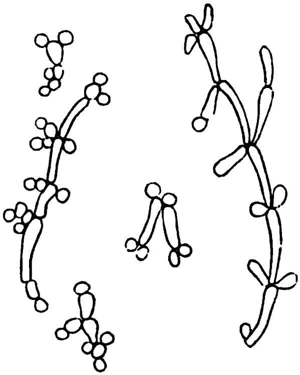

FIG. 12.—*Saccharomyces Pastorianus*. (Pasteur.)

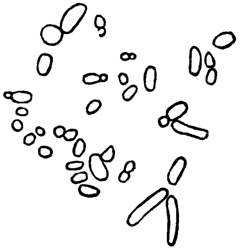

FIG. 13.—*Saccharomyces Pastorianus* after repeated cultivations in nutrient fluids. (Pasteur.)

S. mycoderma forms a thin crust on the surface of fermented or fermentable liquids, frequently appearing spontaneously and multiplying with great rapidity. Its cells are of very various sizes and shapes, ovoid, ellipsoidal, or cylindrical, and from 6μ to 13μ in length by 3μ in breadth. On the surface of a liquid it does not function as a ferment, but when submerged in a suitable fluid, Pasteur showed that it is capable of exciting fermentation. The ascogenous production of spores by the Saccharomyces was discovered by De Saynes in 1868 in this species.

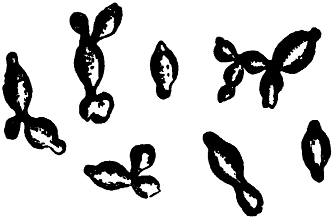

FIG. 14.—*Saccharomyces apiculatus*. (Schützenberger.)

Saccharomyces (*Carpozyma*) *apiculatus* is the commonest form of all those occurring on the outside of ripe sugary fruits, in whose juices it can excite fermentation. It is never found on green fruits, and during the intervals of ripening of the various fruits it develops in the soil beneath the plants. The cells are ellipsoidal, with a small projection at each end, and their greater diameter is about 6μ. The mode of forming ascospores is not precisely similar to that described above, and for this reason it is proposed to remove it from the genus Saccharomyces.

S. albicans is the parasite of the disease known as thrush. It forms pustules on the mouth and throat. The cells are very long, and they excite weak alcoholic fermentation in sugared nutrient solutions.

Other fungi besides the Saccharomyces described above are capable of exciting alcoholic fermentation when submerged in a suitable fluid. One other will be described in this book.

CHAPTER II.

PROTOCOCCUS.

1. REMOVE from a roof-gutter, the side of a water-butt, the trunk of a tree, or similar position, some of the green incrustation to be almost certainly found there. Mount in a drop of water and examine with a high power.

Amongst the mud many spherical green cells will be seen. Measure, and they will be found to be of very different sizes, varying from less than 10μ up to 50μ in diameter. Without reagents it can be seen that there is a fairly stout colourless cell-wall enclosing granular cell-contents, which are mainly of a bright green colour. Sometimes the green colouring matter appears diffused throughout the cell-contents, but generally it can be seen to be confined to portions of the contained protoplasm, forming green-coloured bodies (**Chromatophores**), which are in contact with the inner margin of the cell-wall. Where those do not come in contact laterally, the colourless protoplasm is seen. Frequently a red spot is visible within the cell, and sometimes the red colouring matter is sufficient to give a red tint to a large portion of the contents of the cell, and in this case the organism is generally known as *Hæmatococcus.* Many of the cells are solitary; some are joined together in groups of two, three, or four, with such position relationship as to leave no doubt that they have been formed by the fission of a mother-cell.

2. Treat with solution of iodine in potassium iodide on the slide.

The cell-wall is not stained. The contents of the cell are stained yellowish-brown (reaction of protoplasm). The chromatophores lose their green colour and also take on the brown stain, showing thus their protoplasmic nature, and after appropriate treatment small particles in them take on a blue colour (starch reaction). Cells which are not too deeply stained show a rounded body near their centre which has taken on a deeper brown than the rest of the contents. This is the **Nucleus,** in the centre of which is visible a **Nucleolus.** In a recently divided cell the nucleus is seen near the newly formed partition-wall. In a short time, if a fairly large amount of the reagent has been added, the protoplasm everywhere retreats from the cell-wall (**Plasmolysis**), and allows the latter to be seen with much distinctness.

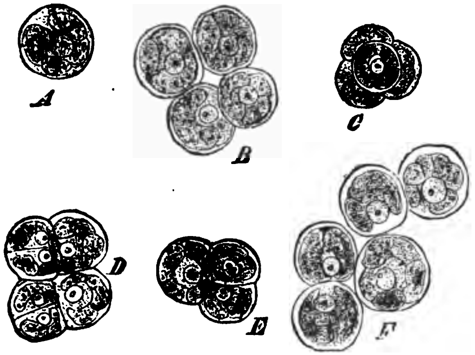

FIG. 15 —*Protococcus viridis* after treatment with potassium-iodide-iodine. The cells show nuclei and chromatophores. (Strasburger.)

Almost all cells—animal and vegetable—have been shown to contain nuclei, and as microchemical methods improve, the list of cells apparently not containing nuclei becomes more and more restricted. The nucleus has been shown to contain at least two distinct substances. One is **Nuclein,** allied to the proteids, but containing phosphorus in its molecule and no sulphur. This is arranged in the form of a network, the meshes of which are filled with another substance not differing much from the general protoplasm (**Cytoplasm**) of the cell. The nuclein framework very readily takes up colouring matters, notably hæmatoxylin. The function of the nucleus in the cell is not well known. It always divides previous to the division of the cell itself, so that newly formed cells each have a nucleus formed by the division of the nucleus of the mother-cell; but since division of the nucleus is not always followed by division of the cell itself, the relation between the two divisions cannot always be one of cause and effect. It is extremely probable that the nucleus is concerned in the processes of cell-nutrition, especially the formation of proteids.

3. Treat protococcus with solution of iodine, and then with 70 per cent. sulphuric acid.

The cell-wall is stained blue, showing that it is composed of normal cellulose. Schulze's solution of iodine in zinc chloride gives the same result.

As already indicated protococcus multiplies by fission. The nucleus divides first, and then a cell-wall is formed, from the protoplasm of the cell, across the mother-cell. Separation and rounding off of the cells ensue, sometimes almost immediately, sometimes not until after further divisions.

4. Watch rain-water butts when the first really warm days of early summer come, or in hot weather when heavy rain falls after drought. At some such time it will be found that the surface of the water is covered with a bright green scum. This remains for some days and then disappears. Remove some, and mount in water.

The field of the microscope is filled with moving green organisms. They are the motile form (**Zoospores**) of protococcus. Focus one which is resting for a moment. It may have a thin coat of cellulose, it will probably be quite naked. Watch for a vacuole in the interior of the green protoplasm, which very slowly increases in size, and then rapidly disappears by the closing together of its walls. Notice that very small particles lying close to the cell are unaffected by the disappearance of the vacuole, which appears thereby to show that nothing either liquid or gaseous is expelled from the organism during that disappearance. The nature of the **Pulsating Vacuole** is unknown.

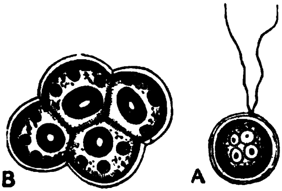

FIG. 16.—*Protococcus pluvialis*. A, motile; B, dividing. (Cohn.)

5. Run in a very little iodine solution under the cover-glass.

The cells are killed and stained. It is then seen that from one end of the slightly ovoid zoospore, which at that point is colourless and transparent, two long and delicate **Cilia** arise. The movements of these cilia probably drive the cell through the water. In the case of the coated motile cells, here also the cilia are processes of the protoplasm which pass through the cell-wall. The red pigment spot is very evident.

After a time the zoospore comes to rest, the cilia are withdrawn, and with the formation of a normal cell-wall of cellulose the cell passes into the resting stage. In fortunate cases the formation of the zoospore may be observed. The protoplasm retreats from the cell-wall, the latter then splits up, and the contents are set free as a motile cell. In some cases, instead of a single zoospore, eight or more are formed from the protoplasm of the cell. In the resting state the cells can resist drying up, and are also unaffected by temperatures below the freezing point of water. In the dry state protococcus forms a bright green incrustation on tree trunks, rocks, and the like, regaining its vital activity when moistened with water.

The growth (increase in size) of the cells of protococcus takes places in a manner similar to that already explained in the case of yeast, and the conditions of its growth are similar. The plant must be supplied with water, food, and oxygen, and be kept at a proper temperature. The optimum temperature for growth is about 25° C.; the minimum is much lower than the minimum temperature for yeast and bacteria, and the maximum is also lower. For healthy, continual growth and reproduction it will appear that light is also necessary.

It will be convenient to examine here the nature of the tension in a turgid cell a little more closely than was possible in the case of the yeast-cell. When liquids are separated by a membrane which both can wet, they diffuse into each other through the pores of the membrane. The liquids may contain solids in solution, in which case if the solids belong to the class of crystalloids they diffuse through the membrane, but if they belong to the class of colloids (gums, starch, and other uncrystallizable bodies), they do not pass the membrane. The direction of the greater diffusion depends on the substance in solution as well as on the nature of the separating membrane.

If the membrane separates two solutions of the same kind, but different strengths, diffusion of the solvent is greater from the weaker to the stronger solution than from the stronger to the weaker, whilst diffusion of the dissolved substance is greater from the stronger to the weaker solution, and continues until the solutions on both sides of the membrane are of equal strength.

6. Arrange an experiment as in Fig. 17. A, B is a funnel which has a piece of bladder tied across its open end. A strong solution of sugar is poured into it, and then the funnel is plunged into a tumbler of water, so deeply that the water in the tumbler stands at the same level, B, as the sugar solution in the funnel.

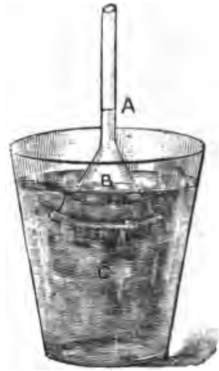

FIG. 17.—Diffusion experiment. C, vessel containing water; A, B, funnel containing solution of sugar.

Diffusion commences, and the level of the solution in the funnel is seen to rise above the level of the water in the tumbler. It is evident that diffusion is greater in amount from the tumbler to the funnel (**Endosmose**) than from the funnel to the tumbler (**Exosmose**). A taste of the water in the tumbler shows that sugar has passed out from the funnel. If at any moment the level of the liquid in the funnel is stationary at A, then the measure of the excess of the inward pressure of the endosmotic current over the outward pressure of the exosmotic current is given by the column of liquid, A, B.

It is evident from this experiment that osmosis is capable of producing a difference of pressure.

7. Arrange an experiment as in Fig. 18. This represents a section of a wooden box, or any other vessel of a convenient shape and size which will hold water. Across it are two partitions of parchment paper or bladder, and for a rough experiment it will be sufficient that the membranes be firmly attached all round to the vessel with marine glue or coaguline and allowed to dry thoroughly. Into the chamber A place

a solution of barium chloride, into C place a solution of sodium sulphate, and into B, the middle chamber, distilled water. Use equal quantities of weak solutions, and let the weight of sodium sulphate used be to the weight of barium chloride as 2 : 3. Allow the piece of apparatus to stand perfectly still.

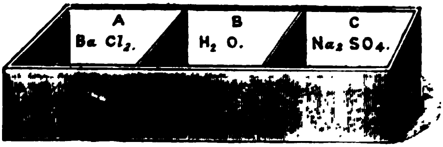

FIG. 18.—Diffusion experiment. A contains barium chloride; B, water; C, sodium sulphate.

Both solutions diffuse into the middle chamber, and where the molecules come in contact a double decomposition is set up, according to the following equation:—

$$Na_2SO_4 + BaCl_2 = BaSO_4 + 2NaCl.$$

The barium sulphate which is formed is a white insoluble powder, and as fast as formed it sinks to the bottom of the chamber B. A continual diffusion thus goes on, by reason of which, in time, A and C lose the salts they held in solution, until at length a mixture of their contents produces no precipitate.

It is evident from this that if by any means a substance is taken out of solution as fast as it diffuses through the membrane, the process of osmosis will continue as long as there is a supply of the diffusible substance, and the removing cause remains efficient. And a little reflection will convince that any change in the diffused substance must produce a continuous diffusion current, because so long as there is change there is impossibility of producing equal strengths of solution on both sides of the membrane.

A protococcus cell may be looked upon as a closed bag with a double wall—the outer of cellulose, the inner of protoplasm. No substance can be nutrient which cannot diffuse through both membranes. The cell-sap which saturates the protoplasm and fills the vacuoles, where there are any, is a thin watery fluid, holding in solution inorganic substances absorbed from the nutrient solution in which the cells live, and organic compounds produced by the metabolism of its protoplasm. There is this resemblance between the protococcus cell and the endosmometer described in Exp. 6. The space or spaces containing cell-sap may be likened to the funnel containing syrup, the nutrient liquid outside the cell to the water in the tumbler, and the bladder to the walls of cellulose and protoplasm. There is an endosmotic current into the cell and an exosmotic current out of it. But many of the substances which enter the cell are changed inside it by the metabolic activity of the protoplasm, and therefore so long as metabolism is active the endosmotic current of nutrient substances continues. If there is any substance in the nutrient solution which the plant does not use in its metabolism, equilibrium between the strength of its solution inside and outside the cell is soon reached and kept. If endosmose is more active than exosmose for any reason, *e.g.* if the walls of the cells expose more resistance to the exosmose of the products of metabolism and of water than to the endosmose of substances in solution in the surrounding liquid, then the pressure inside the cell will be greater than that outside, or, in other words, the cell will be turgid and its walls will be stretched. That this is the case, and that the protoplasm is the resisting agent, has been abundantly proved by experiment.

8. Mount some cells of protococcus in water and place under a microscope. Put a small crystal of common salt or nitre in contact with the water at the edge of the cover-glass, and watch.

The salt enters slowly into solution. The protoplasm is seen to leave the cell-wall, and a clear space appears between the cell-wall and the protoplasm (**Plasmolysis**). The salt can readily pass through the cellulose wall, but not so readily through the protoplasm. If the salt solution is tolerably strong, it withdraws so large a quantity of water from the protoplasm that the latter seems shrivelled up into a small mass in the centre of the cell. Careful measurement shows that the whole cell shrinks slightly as a first result of the action of the salt solution withdrawing water from it. Other vegetable cells, however, show this in a much greater degree (Fig. 19). Thus only weak solutions can pass through the protoplasm and therefore be nutrient.

To sum up. The wall of a growing cell is always on the stretch in consequence of its protoplasm exerting a great resistance to the exosmose of organic products of its metabolism. The increase in size is rendered permanent by the conversion of a peripheral layer of protoplasm into cellulose and its deposition on the cell-wall, and this continues until the cell has attained its full size.

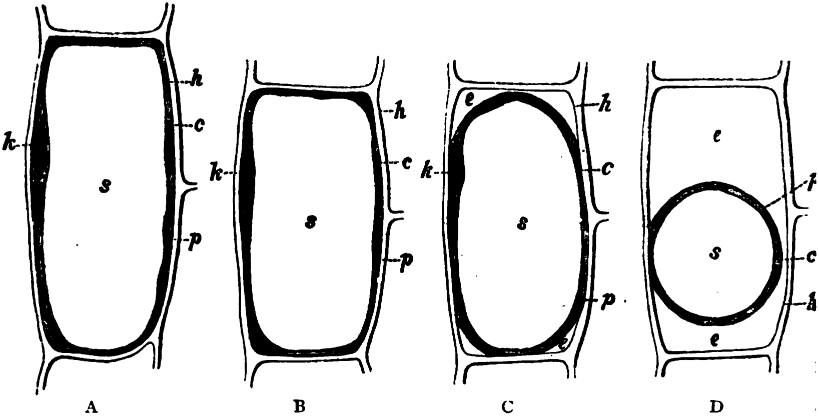

FIG. 19.—A, young parenchymatous turgid cell; B, the same cell in 4 per cent. nitre solution, showing reduction in size; C, in 6 per cent. solution; D, in 10 per cent. solution, showing complete plasmolysis. *h*, cell-wall; *p*, protoplasm; *k*, nucleus; *c*, chlorophyll corpuscles; *s*, cell-sap; *e*, nitre solution which has entered the cell. (After De Vries.)

An analysis of protococcus shows the same gaseous and ash constituents as in yeast and bacteria, with one addition—iron. It must be supplied with these elements in its nutrient solution, and generally in the same form. There are, however, remarkable and important differences between the conditions of life of protococcus and yeast.

Since protococcus flourishes in rain-water, which can contain nothing but what it dissolves from the air and from the dust, it is evident that rain-water is a perfect nutrient fluid for it. It is better, however, to deal experimentally with a nutrient fluid of known composition.

9. Make up the following solution :—Add 300 grains of calcined bone containing tri-calcium phosphate, $Ca_3(PO_4)_2$, to half a pint of water, and then nitric acid to dissolve the bone-ash. Remove excess of nitric acid by adding potassium carbonate, K_2CO_3, until the liquid is slightly turbid. In this operation carbon dioxide is evolved, and the solution becomes charged with it. Then add 170 grains of potassium nitrate, KNO_3, 107 grains of magnesium sulphate, $MgSO_4$, and 46 grains of potassium chloride, KCl, and make up to a quart by distilled water. Take one fluid ounce of this, raise it to a quart by distilled water, and add a single drop of solution of ferric chloride, Fe_2Cl_6. This is Wolff's nutrient solution for green plants. Transfer protococcus to this solution.

It grows and multiplies well in it.

It must be noted that all the constituents of Wolff's solution are inorganic compounds, and particularly that the only compound of carbon present is carbon dioxide, which moreover it absorbs readily from the atmosphere with the other atmospheric constituents. Protococcus, therefore, unlike yeast, is wholly independent of organic compounds for its nutrition, and in particular it is able to do what neither yeast nor most bacteria can do, utilize carbon dioxide as a source of carbon. But carbon dioxide is a fully oxidized compound, which, unlike sugar and other organic carbon compounds, can not only not be a source of energy to the plant, but requires a continual supply of energy to decompose it. And no other constituent of the nutrient solution can supply that energy since they also are fully saturated compounds. But decomposition must take place before constructive metabolism can make protoplasm out of these constituents. Whence, then, comes the necessary energy? In the first place a certain minimum temperature is necessary for the nutrition, growth, and multiplication of protococcus.

10. Remove carefully a layer of mud covered with Protococcus from a roof-gutter, place in water, and expose to sunlight.

It gives off a large number of small bubbles of a gas which appropriate experiments show to be oxygen.

11. Place the same in diffused light.

Less gas, or none at all, is given off.

12. Place and keep it in darkness.

Sooner or later all the protococcus cells die, and bacteria invade and render fœtid the whole mass.

13. Place some in a small bottle of water and cork tightly. Whether exposed to light or kept in darkness, all the protococcus cells die in a few days.

It is clear that light and air are necessary to the life of the plant, which is evidently aërobiotic. Any other green plant placed under the conditions of experiments 10, 11, 12, and 13 would give the same results.

The following will throw some light on the foregoing experiments :—

14. Take a wide glass tube closed at one end. Fill this with water containing a large quantity of protococcus. It will be better if a little soda or seltzer-water be added to it on account of the large quantity of carbon dioxide these mineral waters contain. Invert the tube in a vessel of water and expose to strong sunlight. By this means the bubbles of gas given off by the plant will collect in the upper part of the tube. When a fair quantity has been collected test it with a glowing match, and it will be found to be oxygen.

15. Take well-boiled water and aerate it by passing through it air which has first bubbled through a strong solution of potash. By this means all carbon dioxide is removed from the air. Use this water in an experiment similar to 14.

No bubbles of gas are given off.

The production of oxygen, therefore, in experiment 14 is connected with the presence of carbon dioxide, and more elaborate experiments have abundantly proved that in the presence of sunlight all green plants use up carbon dioxide and give off a volume of oxygen about equal to the carbon dioxide absorbed. We know that protococcus lives under conditions when its only source of carbon is carbon dioxide, and that any increase in its mass means an increase of carbon as well as of other constituents. It is therefore clear that the carbon which disappears is absorbed by the plant, that it retains the carbon and liberates oxygen, and that the energy necessary to effect this decomposition is obtained from the rays of the sun, for if the apparatus be kept in darkness there are no such results.

16. Arrange an experiment as in Fig. 20. A is a lamp-glass closed at its upper end. At its lower end a tube passes through the cork and projects into the glass. The other end of the tube dips into a vessel, B, containing a strong solution of potash. A large quantity of protococcus on moist bark, etc., is put into the lamp-glass, which otherwise contains ordinary air.

The effect is that the potash rises up the tube showing the absorption of carbon dioxide, which could only have been produced by the Protococcus. The diminution in volume of the contained air is owing to the absorption of oxygen by the plant.

In the absence of free oxygen continued life is impossible. The plant exists for a short time, as we have seen saccharomyces exists in a sugared nutrient solution deprived of oxygen, probably because it possesses a store of oxygen which it can draw upon. When this is used up the plant dies.

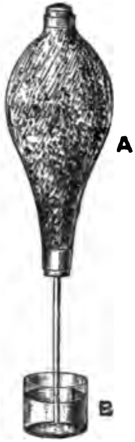

FIG. 20.—Experiment showing the absorption of oxygen and production of carbon dioxide by protococcus. B contains solution of potash.

These experiments make it clear that protococcus in sunlight absorbs carbon dioxide and liberates oxygen, whilst in light or in darkness it absorbs oxygen and liberates carbon dioxide.

Experiments of Engelmann and others have shown that the decomposition of carbon dioxide is most readily effected by the red and blue rays of the spectrum, and it is known that these are the rays which are absorbed by a solution of chlorophyll outside the plant. Engelmann had noticed that *Bacterium termo* is not motile except in the presence of free oxygen. If a filament of a green plant is under the same cover-glass as these bacteria, the latter collect around the filament because it is giving off oxygen. By placing the green plant in the spectrum he found that the bacteria collected most numerously in the red and blue portions of the spectrum about the lines B and C and beyond F, showing that in those parts most oxygen was being evolved.

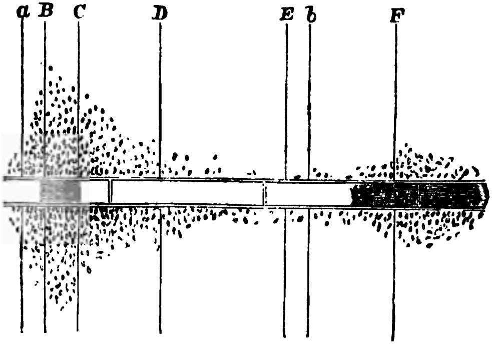

FIG. 21.—A portion of a cladophora-filament in the solar spectrum. The motile bacteria are collected in the greatest number between the lines B and C, that is, at the junction of the orange and red. (After De Vries.)

The agent for the absorption of carbon dioxide and of light is the chlorophyll. In all chlorophyll-bearing plants but the very lowest in the scale of life, the chlorophyll is associated with individualized bodies of a protoplasmic nature, and in these chlorophyll corpuscles the absorbed carbon dioxide is decomposed and its carbon recombined with other constituents to form organic compounds, the first visible product of this constructive metabolism being in almost all cases, starch. Thus the starch grains in the chromatophores of Protococcus are accounted for. Engelmann observed the evolution of oxygen from isolated chlorophyll corpuscles, and this together with the fact that plants containing no chlorophyll, as well as the uncoloured parts of chlorophyll-containing plants, are incapable of decomposing carbon dioxide, sufficiently establishes the proposition that it is this green substance which in the way described renders the green plant independent of organic nutrient substances.

The substance or mixture of substances to which the name chlorophyll has been given has not yet been isolated. "It is a body the molecules of which are in a state of unstable equilibrium, any disturbance of the equilibrium causing the molecules to rearrange themselves so as to form compounds of a more stable character. External influences of an apparently slight character cause it to undergo considerable change, so much so that it may be considered liable to spontaneous decomposition. To this circumstance the difficulty of isolating it and ascertaining its properties and composition is mainly attributable. Chlorophyll is probably a body of complex composition and high atomic weight, such as might be expected in the case of a body playing so important a part in the vegetable economy." It is insoluble in water, but is easily dissolved out of the plant by alcohol, ether, chloroform, carbon disulphide, and other such solvents, but always in company with other substances from which it has not been separated. It certainly contains carbon, hydrogen, oxygen, and nitrogen, and in the ash of the residue from the evaporation of a solution are always found ferric oxide, and calcium, and magnesium phosphates. It is not thought, however, that the iron is a constituent of the chlorophyll, but of the substances accompanying it, although it is known that chlorophyll is not formed in plants which are not supplied with iron.

A solution of chlorophyll has a bright green colour, but shows a red fluorescence. As has been stated, its absorption spectrum shows bands in the red, blue, and violet portions of the spectrum.

The exact share taken by chlorophyll in the assimilation of carbon dioxide is not certainly known. It may be that besides absorbing the necessary energy in the form of light, it acts as a carrier of carbon dioxide, holding it loosely, but in a condensed form, and transferring it to its associated protoplasm which effects the actual decomposition, the chlorophyll acting like the hæmoglobin of blood as a carrier of oxygen.

From the carbon dioxide thus assimilated, and the mineral substances absorbed, the plant builds up proteids, and from the proteids are formed protoplasm. All constructive metabolism is independent of light except the initial decomposition of carbon dioxide.

As in the case of yeast, and indeed of all living things, destructive metabolism is continuous whether constructive metabolism is going on or not. By it the protoplasm is broken down into simpler bodies. Some of the products of destructive metabolism are held in reserve, to be again used in the construction of protoplasm; others are mechanically useful, and others again are excreted. Cellulose is known to be a product of the destruction of protoplasm, and starch and oil are almost certainly so. The destructive metabolism of protoplasm is accompanied by the absorption of oxygen—carbon dioxide, water, and amides being formed. This absorption of oxygen and giving off of carbon dioxide is known as **Respiration,** and heat is set free in the process.

The course of metabolism in a green plant may thus be represented—

METABOLISM.

Constructive.	*Destructive.*
Carbon dioxide, water, nitrogen compounds, mineral compounds	Protoplasm
Carbohydrate and amides	Waste and plastic products and kinetic energy.
Proteids	
Protoplasm and potential energy.	

We can now strike a balance-sheet of income and expenditure of the green plant—protococcus and others.

INCOME.	EXPENDITURE.
Matter: Food-substances.	*Matter:* Carbon dioxide, water. Excess of cell-formation.
Energy: Radiant (light and heat).	*Energy:* Heat evolved. Work done in growth and movement. Potential energy of organic compounds formed

BALANCE IN FAVOUR OF THE GREEN PLANT.

Matter: Excess of tissue-formation.
Energy: Potential energy of organic compounds formed.

CHAPTER III.

BACTERIA, OR SCHIZOMYCETES (FISSION FUNGI).

1. MAKE a weak infusion of almost any vegetable or animal substance, *e.g.* hay, green leaves, potatoes, carrots, turnips, peas or beans, meat, in hot water; allow to cool down to from 30° C. to 35° C., and keep it at that temperature exposed to air.

In the course of a day the fluid becomes turbid, and on the surface a thin pellicle appears, which soon covers the whole surface of the liquid. The pellicle may be semi-transparent or dead white and opaque, with yellow, brown, red, and other coloured patches on it. It may be thin and tough, or crisp and easily broken through.

2. Make such an infusion in a flask; boil three or four times, with intervals of a few hours between consecutive boilings, and with the neck of the flask stopped with a plug of cotton wool. Keep this at a temperature of about 30° C.

No pellicle appears on the surface, although the cotton wool allows air to pass freely into the flask. It, however, filters all solid matters, even the smallest particles, from it.

3. Make an infusion of hay in warm water. Boil for an hour in a flask stopped with cotton wool, cool down to 30° C., and keep at that temperature.

A pellicle forms on the surface. An hour's boiling is not sufficient to prevent its formation.

4. Arrange another experiment as in 2, but leave the flask open and exposed to the air.

Sooner or later a pellicle is formed on the surface. It is obvious that the exciting cause in this case must come from the air.

5. Place pieces of cooked vegetables, such as boiled potatoes, carrots, turnips, greens, exposed to the air. Cover merely with a glass to exclude dust, and keep at a temperature of 30° C.

In a day or two they will appear covered with variously coloured patches—white, red, pink, yellow, brown—of varying consistency, watery-looking and slimy, or dry on the surface, although gelatinous. Some are small dome-shaped elevations, others are irregularly shaped patches, and others still confluent slimy masses.

6. Lay a small portion of a pellicle on a slide in a drop of water, cover with a cover-glass and examine with the highest power at command.

The pellicle may appear to be striated, the striation being due to sometimes small rod-shaped, sometimes spherical bodies arranged in parallel rows. Or it may show motile rodlets from the substratum which will be seen darting about with great velocity, at the same time revolving on their own longitudinal axes. Or it may chance to contain very long thin rods which are sometimes bent upon themselves at sharp angles; or spirally twisted motile rodlets, some but slightly and others much coiled. The gelatinous patches on the cooked vegetables show bodies of the same description. They are **Bacteria**, or **Schizomycetes**, the latter word meaning **Splitting** or **Fission Fungi**, although there are many reasons for believing the bacteria are not true fungi. Although it is certain that many which appear similar in shape and size are really different species, it is convenient to adopt form-names for them. The chief forms of bacteria are—

Micrococci	Small spherical cells.
Macrococci	Larger spherical cells.
Bacteria	Short rods.
Bacilli	Longer rods.
Leptothrix	Long unbranched filaments.
Spirochæte and Spirillum	Spiral forms with narrow coils.
Vibriones	Spiral forms with distant coils.

It will be shown in the sequel that one and the same species can assume several of these forms at different times.

7. Spread out a small portion of a pellicle in a drop of

water on a cover-glass, and allow to dry. When quite dry pass the cover-glass, pellicle upwards, rapidly through a flame a few times, taking care to keep the thin layer of bacteria upward. The heat coagulates and fixes in position pellicle and cell-contents. Lay the cover-glass in solution of gentian violet, vesuvin, or methyl blue for five minutes, wash well with water, allow to dry, and mount in Canada balsam.

The aniline colours stain bacteria intensely, and none more rapidly and deeply than those above named, especially gentian violet. The cells are seen far better when stained than when unstained.

In nearly all cases much smaller in diameter than yeast-cells, some of them being the smallest known living things—and many much less than 1μ in diameter—they lie at the very limit of distinct vision even with the highest powers of ordinary microscopes. For their complete examination high power immersion objectives, with special arrangements for illumination, such as the student will not often have at his command, are required.

The bacteria are bodies of a low refractive power, consisting of a cell-wall which encloses homogeneous or slightly granular protoplasm. The outer layer of the cell-membrane is swollen and gelatinous, and in many cases contiguous cells are united by it, the whole forming the pellicle or slimy mass which holds together the colony. Such a coherent colony is known as a **Zooglœa.** In some bacteria the membrane is composed of a carbohydrate very closely allied to fungus-cellulose; in others—the bacteria of the putrefactive processes—it is of an albuminous nature; and to this latter substance the name **Mycoprotein** has been given. The cell-wall is extensible and elastic, rarely coloured, although in some of the red, blue, and yellow colonies the colour appears to belong to the cell-membrane rather than to the cell-contents. It is of course pervious to liquids and substances in solution like the cell-wall of yeast, since increase of the cell in size must generally be accompanied by absorption of food-stuff.

The cell-contents are in most cases colourless protoplasm in which are imbedded granules of fat, particles of a proteid nature (microsomata), and in some species small crystals of sulphur. In one species, *Beggiatoa roseopersicina*, a red colouring matter, which Lankester has examined the nature of and called **Bacteriopurpurin**, is diffused throughout the protoplasm. Three species are known—*Arthrobacterium viride*, *Bacillus virens*, and *Arthrobacterium chlorinum*—which contain chlorophyll, and which are therefore able to decompose carbon dioxide. Nuclei are not known even in the largest forms. In some species immediately before spore-formation the contents of those cells about to form spores give a violet colour with iodine, showing that there is a starchy substance present.

It has been already stated that some bacteria are actively motile. The mechanism of movement is not known. It is known, however, that many are possessed of vibratile cilia, extremely thin threads which lash backward and forward through the water; but whether such cilia are processes of the cell-membrane or of the cell-contents is not known. And since many motile bacteria have no cilia, it is not necessary to suppose that cilia are essential to movement.

The conditions of nutrition and development of the schizomycetes correspond closely to the conditions of nutrition and development of the yeast fungi, and, like the latter, their most characteristic feature is, that whilst absorbing but a comparatively small amount of the nutrient substratum for their growth and reproduction, they effect profound decompositions in the mass by their fermentative actions. An analysis of bacteria shows the same gaseous and ash constituents as are found in yeast, and it therefore follows that its food should contain the same elements as Mayer's nutrient solution. There is this difference, however, in the conditions of life of yeast and schizomycetes, that whereas the former flourishes best in acid solutions, the growth of the latter is arrested by acids and it does best in neutral or slightly alkaline solutions. Mayer's solution on this account is not very well suited for the cultivation of bacteria, as it is distinctly acid. The schizomycetes do not convert sugar into alcohol, and this constituent is not in general necessary for a complete nutrient medium. The best is a solution containing peptones and mineral constituents, and infusions of animal and vegetable substances like those above described are of this nature.

In or on such a solution, as well as on a solid substratum of organic matter, the plant is living the life of a **Saprophyte,** *i.e.* it is getting the bulk of its food in the form of complex organic substances not forming part of living tissues. Some schizomycetes are capable of spending part of their lives in the interior of living animal tissues or the cavities of living animal bodies, and such are **Facultative Parasites.** A few are known to be **Obligate Parasites**—incapable of development outside living bodies. The schizomycetes known to be parasitic on vegetables are extremely few, and the reason is probably this. They are not capable of piercing cell-walls, and those intercellular spaces of plants which open to the exterior contain no nitrogenous compound capable of affording them nourishment. But after the attacks of parasitic fungi, which break down cell-walls, as well as after application of moist heat (cooking), which breaks up the cells, abundance of bacteria will be found in them.

Both aërobiotic and anaërobiotic species of bacteria are known.

The optimum temperature for the growth of most schizomycetes is from 30° C. to 35° C. The minimum is somewhat higher than that for yeast, and the maximum is also slightly higher. The optimum temperature for the germination of spores varies from 30° C. to 40° C. in the different species, and the minimum is not much below 20° C. for most, and above that temperature for some species.

Whilst the vegetative forms are killed by moist heat at about the same temperature which kills yeast cells, the spores of the endosporous species are more resistent to heat and to desiccating agents than any other known organism. Spores of *Bacillus anthracis* are not killed by being placed in absolute alcohol, nor by three years' drying in the air. Boiling in nutrient solution for fifteen minutes does not destroy them. The spores of *Bacillus butyricum* and *Bacillus megaterium* have equal powers of resistance. Pasteur says that some spores can stand a temperature of 130° C. Spores of *Bacillus* *subtilis*, the bacillus of hay infusions, germinate better and more rapidly after boiling (Exp. 3). On the other hand, Frisch found that bacteria of putrefaction retained their power of development after having been cooled down to − 111° C., and the bacillus of typhoid fever has been found still alive in ice which has remained frozen for 103 days. The reasons for the successive boilings and coolings of Exp. 2 are now seen. Boiling the fluid killed the vegetative forms and hastened the germination of the spores, the vegetative forms thus produced being killed at the next boiling. At the end of the experiment the liquid is completely sterilized.

The advantages to the plant of having its spores so resistent to extremes of temperature and to desiccating agents are obvious.

In their methods of multiplication the schizomycetes differ fundamentally from the saccharomycetes. As their name implies, the former multiply by the fission or splitting of cells. A cell-wall is formed across a pre-existing cell, and the products of the division are in all cases similar in character to the cell from which they are formed. Very frequently a rod or thread-like chain of cells is formed in this manner, the cells remaining connected at their extremities, and in this case, which is perhaps the most common, the successive division walls are in parallel planes. Frequently, however, the division walls are not formed in parallel planes, and the result is an irregular mass of cells as we find on solid substrata. It is not easy to see that these rods are really divided by partitions. If, however, such a large species as *Bacillus megaterium* be treated with alcoholic iodine solution, the division walls appear clearly on account

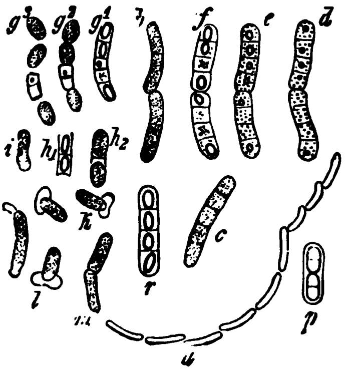

FIG. 22.—*Bacillus megaterium*. *a*, motile chain of rods; *b* and *c*, rods; *p*, a quadricellular rod; *d–f*, rods forming spores; *r*, rod with ripe spores; *g–l*, germination of spores in a nutrient solution. (De Bary.)

of the staining of the protoplasm and its withdrawal from the cell-walls.

The endogenous formation of spores—one in each cell—has been observed in a large number of schizomycetes, and these are classified together as **Endosporous Bacteria**; the others, **Arthrosporous Bacteria,** so far as is known, form no spores in their cells, although the individual cells of a series frequently separate and become the starting members of new series.

As an example of endosporous bacteria may be sketched the life-history of *Bacillus subtilis*, the form which appears in hay infusions. The pellicle consists of parallel strings of the bacillus united by the gelatinous outer membranes of the cell-walls. Treatment with iodine or staining with gentian violet enables us to see that the strings are composed of rodlets united end to end. These are constantly dividing, but a magnification of at least 1000 is necessary to show the actual process of segmentation of the cell. If the plant be deprived of nourishment it proceeds to form spores. A highly refractive particle appears in a cell and rapidly increases in size, whilst the general protoplasm of the cell disappears, until at length the completed spore is seen to lie in the mother-cell. The spore is set free by the disappearance, in the course of a

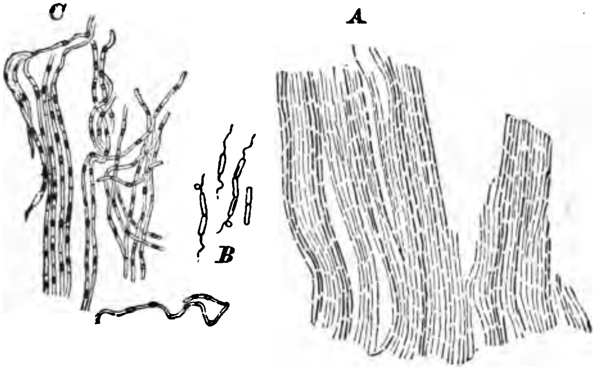

FIG. 23.—*Bacillus subtilis.* A, the pellicle; B, swarming rodlets; C, spore formation.

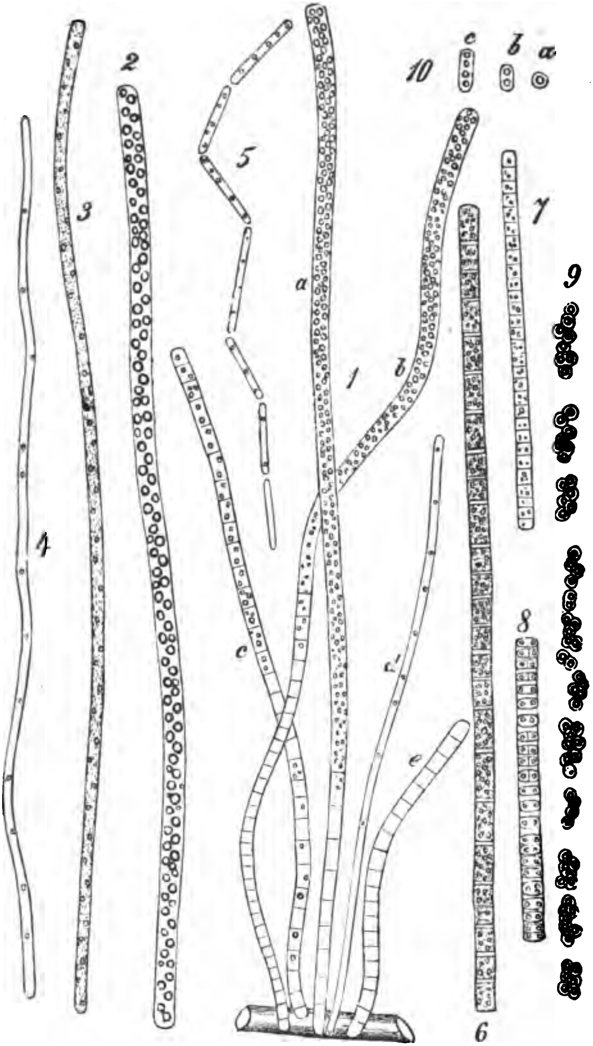

FIG. 24.—*Beggiatoa alba.* 1–4, filaments; 5, filament breaking up; 6–8, filaments showing divisions on treatment with methyl violet; 9, a filament broken up into spores; 10, motile spores. (Zopf.)

few hours, of the cell-wall of the mother-cell. It germinates readily in a nutrient fluid at about 35° C., more readily if first boiled for a short time. In germination the spore-wall splits on one side and a germ-tube protrudes transversely. This increases in length whilst one end of it still remains in the empty spore-membrane. When first formed the rod is motile and furnished, it is said, with cilia; it divides and subdivides whilst still moving rapidly through the liquid. When the free oxygen of the substratum has been exhausted, it rises to the surface, loses its power of movement, and the formation of a pellicle is commenced.

Beggiatoa alba is a good example of the arthrosporous bacteria. It forms tufts of filaments attached at one end to fixed objects in sulphur springs, dirty water, and water containing decaying algæ, especially Characeæ. It may generally be readily procured from pieces of vulcanized rubber allowed to stand in water. The threads vary in thickness from 1μ to 5μ, and they contain numerous strongly refractive sulphur granules which hide the

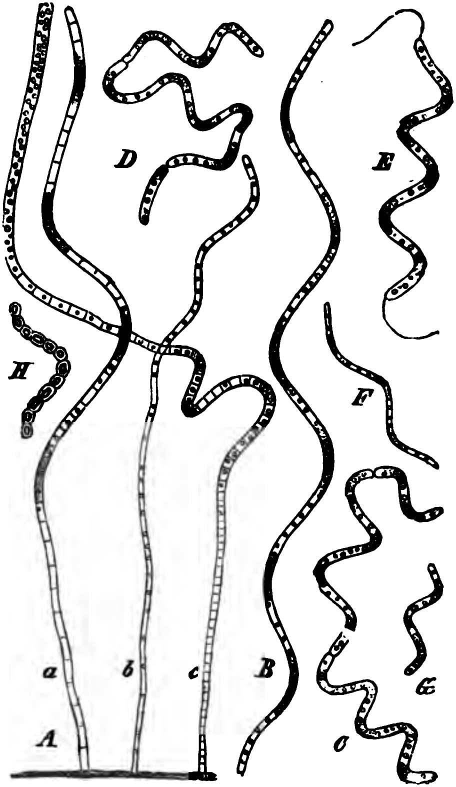

FIG. 25.—*Beggiatoa alba.* A, attached filaments; B–H, portions of spiral filaments; E, filament with cilia. (Zopf.)

division walls until the sulphur is dissolved out with carbon disulphide, or until the threads are stained. The constituent cells of the threads readily separate from each other, round themselves off, and become actively motile cocci, which ultimately come to rest, attach themselves, and develop again into rods, or threads, or form jelly-like patches. Occasionally division of the cells of thick filaments into four daughter-cells by division walls in planes at right angles to each other may be observed (Fig. 24, 8). Motile spirals are sometimes developed, and even the attached threads are frequently spirally bent and show slow movements of their free ends.

The effects of the schizomycetes on the substratum are many and varied, and depend as well on the species living and multiplying in it as on the nature of the substratum itself. These effects are not wholly analytical, as is the case in the alcoholic fermentation by yeast, but with some species of schizomycetes synthetic changes also occur. They are all true fermentative processes, but whereas the term fermentation is commonly applied to the changes brought about in the carbohydrates and allied compounds by the living activity of organisms, the decomposition of albuminoids under their influence is generally termed putrefaction, not because there is any reason to suppose that the changes are essentially different in character from those which the carbohydrates undergo, but because of the fœtid character of the products.

Arthrobacterium (*Mycoderma*) *aceti* (mother of vinegar), a micrococcus, in the presence of abundance of free oxygen, brings about the oxidation of ethyl alcohol to acetic acid, and this is made use of on a large scale in the manufacture of vinegar.

$$\underset{\text{Alcohol.}}{C_2H_6O} + O_2 = \underset{\text{Acetic acid.}}{C_2H_4O_2} + H_2O$$

The oxidizing action of the plant becomes too vigorous when well supplied with food, and the acetic acid is further oxidized to carbon dioxide and water. Complete combustion of the first products of fermentation are also brought about by other bacteria, *e.g.* *Bacillus subtilis* and *Bacillus Megaterium*.

The **Viscous** or **Mannitic** fermentation of sugar is effected by a micrococcus of diameter 1μ to $1\cdot4\mu$. Wines or other fermented fruit juices such as cider are subject to this fermentation, when the unaltered sugar which they contain is converted by the fermentative action of the bacterium into dextrine, mannite (a natural sugar), carbon dioxide, and water. The liquid becomes viscous.

When urine is exposed to the air, sooner or later it loses its acid reaction and becomes alkaline in consequence of the urea which it contained at first having been converted into ammonium carbonate. A pure solution of urea remains unaltered if exposed to air, because such a solution does not contain the nutrient matters necessary for the support of life. The change is one of hydration—

$$\underset{\text{Urea.}}{CH_4N_2O} + 2H_2O = \underset{\text{Ammonium carbonate.}}{(NH_4)_2CO_3}$$

and is brought about, as Pasteur and Van Tieghem have shown, by a micrococcus of extremely small size, not more than $\cdot2\mu$ in diameter.

The **Lactic** fermentation is wholly an analytical change, in which milk-sugar and grape-sugar are converted into lactic acid.

$$\underset{\text{Grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{Lactic acid.}}{2C_3H_6O_3}$$

The determining cause of the lactic fermentation was found by Pasteur to be an anaërobiotic bacillus. Milk and sugary solutions containing albuminoids are subject to this fermentation. When milk or such a solution "turns sour" the milk-sugar or cane-sugar first suffers hydrolysis and conversion into grape-sugar, as in the case of the alcoholic fermentation by yeast. The lactic fermentation does not continue until the whole of the sugar is converted, unless the acid is neutralized as fast as formed, on account of the inability of the ferment to thrive in acid solutions.

Lactic acid and substances which undergo the lactic fermentation are capable of being converted into butyric acid under the influence of an anaërobiotic motile schizomycete, *Clostridium (Bacillus) butyricum*, about 2μ in diameter up to

20μ in length. Sugars and other carbohydrates, tartaric, citric, and other acids are examples of the compounds giving rise to butyric acid under the action of this bacillus. Free oxygen destroys the power of *Clostridium butyricum* to bring about this change.

The decomposition of animal tissues in the process of putrefaction has been investigated by Pasteur and many others, who have shown that it consists of a complete series of fermentations and combustions, brought about by the vital activity of anaërobiotic vibrios and aërobiotic bacilli, mucors, and other organisms. Putrescible matters first nourish some forms, usually *Bacterium termo*, which uses up the free oxygen. Then a film of bacteria and mucors forms on the surface, which shields the substratum from the atmosphere, whilst vibrios flourish beneath. The proteids suffer oxidative decomposition into the amides—Leucin and Tyrosin. Then follows a series of successive fermentations on more and more simple products. Acetic, propionic, butyric, and other volatile fatty acids are formed; ammonia, compound ammonias, sulphuretted hydrogen, and other putrid gases are given off, with carbon dioxide, hydrogen, and nitrogen, the more complex compounds formed by the vibrios beneath being oxidized to simpler compounds by the bacteria and mucors on the surface.

It is now a matter of common experience that the necessary conditions to prevent putrefaction in animal matters are those which render the medium unsuitable for the growth and multiplication of bacteria. The long-held opinion that albuminoids and compounds allied to them are necessarily very unstable compounds has been shown to have no foundation in fact. Meat can be preserved for any length of time if it be kept at the freezing temperature of water, because at that temperature bacteria cannot develop, or if after all germs in it have been destroyed by heat it be kept from the floating germs of the atmosphere, or if it be completely dried, or if it be covered or impregnated with any antiseptic compound such as common salt. The system of antiseptic surgery, by which a wound is shielded from floating germs by septicides, has enormously reduced the mortality following serious surgical operations.

It has been quite clearly proved that many of the parasitic schizomycetes excite disease in their host by virtue of their parasitic properties. In anthrax, a disease chiefly affecting the lower animals, erysipelas, recurrent fever, fowl-cholera, silk-worm disease (*Flacherie*), consumption, and many others, the exciting causes have been shown to be species of schizomycetes. The cells of the bacillus of anthrax, *Bacillus anthracis*, are about 3μ in length by 1μ in thickness, and are connected together into straight rods of various lengths. When introduced into the blood of an animal they multiply rapidly, and the result to the animal is usually fatal, although some are far more susceptible than others to the attacks of this parasite. Whilst in the body of the animal *B. anthracis* does not form spores, but rods expelled from the animal in its fæces produce spores on the pasture-ground, and these are doubtless eaten by other animals, thus spreading the disease. *B. anthracis* is thus a facultative parasite. It is capable of passing its whole life outside the animal body, but it is also capable of passing a portion of it inside the animal body. When cultivated in a neutral nutrient solution, with plenty of free oxygen, and at a high temperature, it multiplies freely but does not form spores, and its destructive properties undergo such a weakening that it can no longer excite disease in even the most susceptible animal. Inoculation of an animal with this attenuated form gives it immunity against anthrax, just as inoculation with *Micrococcus vaccinæ* (*Vaccine lymph*) is a protection from smallpox. The protection thus afforded is due to the production of a soluble substance by the germs of the disease. This substance may be obtained free from germs by killing them by heat or removing them by filtration through porcelain, and it has been shown that such solutions have the power of protecting the vaccinated animal from septicæmia, anthrax, typhoid fever, diphtheria, tuberculosis, and other diseases if the germs of the disease happen to enter the blood or tissues by chance infection.

CHAPTER IV.

MUCOR.

1. DAMP a piece of bread, and put it under a glass so as to keep it in a moist atmosphere. In a day or two it will be found covered with white fleecy threads, and on the ends of some of them appear small black knobs large enough to be seen with the naked eye. The threads are probably hyphæ of *Mucor mucedo*, or of some other species of *Mucor*, and the black knobs are **Sporangia** containing **Spores.**

2. Make a damp chamber for the microscope by taking a piece of cardboard the size of a glass slide, cutting a round hole rather smaller than a cover-glass in the middle of it, and binding it on the slide at each end with a piece of string. Then soak it in water until soft. Other and better damp chambers can be bought or made, but this is sufficiently good for our purpose. Place a drop of water in the middle of a cover-glass, add to it a very small quantity of the liquid from some preserved fruit such as ordinary household jam, place in it one of the Mucor sporangia, and invert it on the cardboard so that the drop hangs freely in the chamber. If the cardboard be wetted occasionally there will be no evaporation from the drop worth speaking of. Place under as high a power of the microscope as can be brought to bear upon it, and examine frequently the course of germination of the spores.

The wall of the sporangium dissolves as soon as placed in water, and the spores will be seen to lie in a thin mucilage. Each spore is slightly oval, about 10μ in its longest diameter and 8μ in its shortest. It has a very distinct doubly outlined wall of fungus cellulose, and granular protoplasmic contents. In the course of a few hours the spore increases considerably in size, the double outline of the wall becomes less distinct, and a large irregularly shaped vacuole forms within it. Then the wall bulges slightly at one or more points, and at these points the outer wall is pierced through, and the inner wall protrudes as a cylindrical germ-tube with hyaline protoplasmic contents. The development is now very rapid, the germ-tube frequently doubling its length in less than an hour, branching and spreading in all directions. The threads thus produced are known as **Hyphæ.** For a considerable time no division walls are formed in the branched tube, and a layer of protoplasm remains in and clothes the wall of the spore, rendering it a permanent portion of the plant.

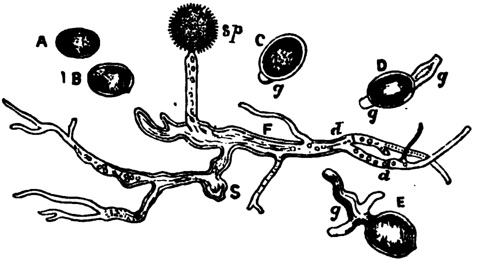

FIG. 26.—*Mucor mucedo*. A, B, C, D, E, successive stages in the development of the spore in a nutrient solution; F, mycelium developed from a single spore (the hyphæ are not quite normal on account of the mycelium being submerged); S, the spore; *sp*, sporangium; *g*, germ-tubes; *d*, division walls.

Sown on the surface of damp bread, fruit, dung, or such a nutrient solution as Pasteur's, with either ammonium tartrate or peptones, the spores germinate as just described, and, interlacing their hyphæ, form a dense **Mycelium,** which soon covers the whole surface, clinging closely to it and penetrating into it. Soon branches of the hyphæ rise erect into the air to the height of an inch or more. These are **Sporangiophores** (sporangium-bearers), and are stouter than the majority of the mycelial hyphæ. If equally illuminated on all sides, they grow vertically upwards and are on that account said to be **negatively geotropic.** If the light is stronger on one side than on the other then they incline towards the light, and are therefore said to be **positively heliotropic.** The mycelial hyphæ are distinctly **positively geotropic.** In our damp chamber cultivation it will be found that the sporangiophores which arise from the mycelium, for a portion of their growth, remain immersed in the nutrient fluid, and then bend downward to grow out of the hanging drop. The tendency of an organ to grow away from moisture is called negative hydrotropism, and the sporangiophore is accordingly **negatively hydrotropic**, but at first its negative geotropism is more potent than its negative hydrotropism, whilst later the reverse is the case.

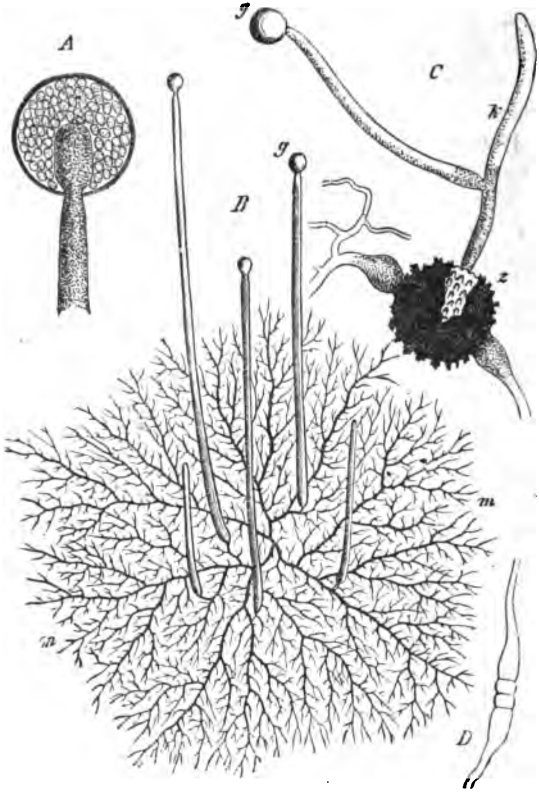

FIG. 27.—A, C, and D, *Mucor mucedo*; A, sporangium in optical longitudinal section; C, zygosphore; *z*, *k*, germ-tube; *g*, sporangium; D, conjugation; B, *Phycomyces nitens*; *m*, mycelium; *g*, sporophore. (Brefeld.)

Up to the time of the formation of the sporangiophore the whole mycelium consists of a simple, unseptate, profusely branched cell the centre and origin of which is the spore, but now the sporangiophore swells at its apex into a sphere, a division wall forms beneath it and cuts off the sporangium from the sub-aerial hypha which bears it. This hypha, however, continues growing in *Mucor mucedo* in such a manner as to protrude into the cavity of the sporangium a club-shaped portion, the **Columella.** Its growth is then ended. Other division walls now appear in the mycelial hyphæ, but at very irregular intervals.

3. Mount a portion of the mycelium in water, and examine with a high power.

Note the tubular branched hyphæ with rare division walls, and the colourless granular protoplasm with frequent large vacuoles.

4. Mount a portion of the mycelium in one per cent. solution of osmic acid.

Most of the granules, some of which in the older hyphæ are very large in proportion to the size of the hyphæ, are turned brown. This shows that they are particles of fat.

5. Mount another portion in solution of iodine in potassium iodine.

The walls are unstained; most of the contents show the yellow protoplasm reaction. Places here and there show a strong reddish-brown colour. This is the reaction for **Glycogen,** a carbohydrate nearly allied to starch, and which is occasionally met with in the fungi. Nothing takes on a blue colour, showing that starch is not present. Proper treatment brings out the fact that a large number of very small nuclei are imbedded in the protoplasm (Strasburger).

6. Treat another portion with iodine and then sulphuric acid.

The walls of the younger portions of the mycelium, as well as those of the sporangiophores, are coloured blue. We have here, therefore, to deal with ordinary cellulose.

Immediately after the sporangium is cut off from its sporangiophore by the division wall, the protoplasm of the former is divided into a very large number of parts by the formation of very delicate, clear partition walls, and each portion of protoplasm thus isolated rounds itself off and takes on a stout cellulose coat. The wall of the sporangium, which is covered on the outside with small radiating crystals of **Calcium Oxalate,** becomes so altered that, placed in water, it immediately dissolves and liberates the enclosed spores, the partition walls between them swelling up into a mucilage which for a time holds the spores together. The wall of a young sporangium immersed in water does not dissolve. A portion of the base of the sporangial wall remains attached to the base of the columella, and is called the **Collar.** The sporangiophore, with its columella and collar surrounded by spores, are all that are seen when a ripe sporangium is mounted in water and examined. The spores require no resting period, but under proper conditions of nutriment, oxygen, and temperature, they germinate immediately.

Generally the development of *Mucor mucedo* ends with the production of spores, but under certain conditions other reproductive bodies are formed on the mycelium. Two branches, similar in size and appearance, but different in origin, arise and approach each other until their points are in contact. Division walls cut off the apical portion of each,

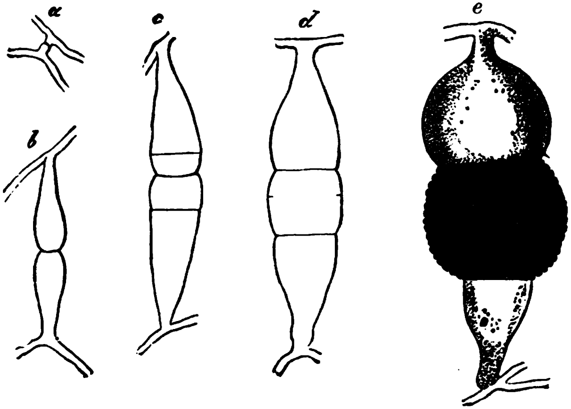

FIG. 28.—*Rhizopus nigricans.* *a–e,* successive stages in the formation of a zygospore. (De Bary.)

the wall between these apical portions disappears, and their contents unite to form a **Zygospore.** The process is the sexual one of **Conjugation,** the conjugating cells are **Gametes,** and the remaining portion of each gamete-bearing hypha is named a **Suspensor.** Zygospores are most freely produced on a substratum of dung in spring. Strasburger says that they can be easily obtained at any time by sowing spores of mucor in concentrated plum juice mixed with from ten to twenty per cent. of ethylic alcohol in a damp chamber. The ripe zygospore is coated with a thick black **Exospore** (outer coat), covered with warty protuberances, and within this is a delicate **Endospore** (inner coat). Very many species of the **Mucorini** produce no zygospores, and the zygospores of some which do are not capable of germination. In all cases the number of spores produced is far in excess of the number of zygospores.

The zygospore is not capable of immediate development. It has a resting period of several months. Then, under favourable conditions of temperature and nutriment, the exospore bursts and the endospore protrudes as a germ-tube —a pro-mycelium—which immediately produces an ordinary sporangiophore (Fig. 27). The sexually produced zygospore invariably produces asexual spores, but many generations may pass before sexual organs are again produced.

Two species of Mucor, viz. *M. tenuis* and *M. fusiger*, as well as others of the Mucorini, are known to produce gametes which do not conjugate, but yet form reproductive cells similar in appearance and equal in capability for germination to zygospores. These are known as **Azygospores.** Such spores are said to be **parthenogenetically** formed.

Mucor mucedo is a saprophyte. It is one of the commonest of all the moulds, being found on the most diverse nutrient substrata. The mycelium, lying in or on the substratum, functions as a root absorbing nutrient matters for its own maintenance as well as for that of the sporangiophore, which being in the air, is not an absorbing organ for solids in solution. The entrance of food compounds into the hyphæ, and the course of constructive and destructive metabolism in the plant are as have been already described for yeast. One very striking illustration of the endosmotic activity of the absorbing mycelium of *Mucor* is given by the fact that very frequently small drops of water are found on the sporangium. This water, with nutrient substances in solution, is absorbed by the hyphæ of the mycelium with so much energy that it is forced out through the wall of the sporangium. It is hence evident that the protoplasm there does not exert so much resistance to the exosmose of water as does the protoplasm of the mycelial hyphæ, and we have thus an instance of a vegetative cell whose protoplasm at two different points has different osmotic properties.

Most of the Mucors are saprophytes, and, like other saprophytes, their most suitable nutrient solution is one containing peptones. They can, however, make use of both ammonia compounds and nitrates as sources of nitrogen. Since they possess no chlorophyll they cannot decompose carbon dioxide. A slightly acid solution is better for them than a neutral one.

Mucors are known which are parasitic in warm-blooded animals, and *Mucor stolonifer* and *M. racemosus*, ordinarily saprophytes, can make their way through the tissues of sound juicy fruit once the rind is broken, and set up alcoholic fermentation of their sugary juices, thereby rotting them.

Under normal conditions of growth, the Mucors obtain their oxygen for respiration in the free state from the air or from the solution in which the mycelium lies, and are therefore aërobiotic. If, however, the mycelium of some of them be immersed in a sugared nutrient solution which contains but little free oxygen, they decompose the sugar, as yeast does, bringing about a regular alcoholic fermentation. When this is the case, the development of the plant and the

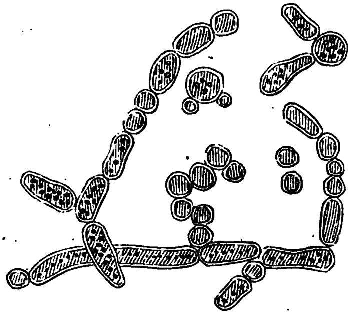

FIG. 29.—*Mucor mucedo* growing submerged with insufficient air. (Pasteur.)

structure of the mycelium is not normal. Instead of producing long, branched, and in the main unseptate hyphæ, it forms short globular cells which have the power of producing similar cells by sprouting. This form is so much like yeast—except that the sprouts are very much larger, even up to 40μ in diameter—that it has received the name *Mucor yeast*. These sprouting cells, germinating with plenty of free oxygen, produce a normal mycelium. Pasteur showed that *Mucor racemosus* causes a fairly active fermentation in a sugared solution, but the fermentative activity of *Mucor mucedo* is small. Living in or on a sugared nutrient solution and supplied with plenty of free oxygen, no more sugar is decomposed than is necessary to supply the plant with carbonaceous food, or, at any rate, if alcohol is produced under these circumstances, it is burnt by the plant as fast as formed (Pasteur).

CHAPTER V.

PENICILLIUM.

ONE of the commonest of all the moulds, *Penicillium*, is found living on the most diverse substrata where only traces of moisture and nutriment are to be found. The mucor on the piece of damp bread used for the last lesson is soon crowded out and replaced by a green mould, which rises but a slight distance above the surface. This is almost certainly *Penicillium crustaceum*, or some other species of penicillium. On a clear substratum, *e.g.* on the surface of a nutrient liquid, it spreads in all directions from a centre, sending its mycelium into the liquid and raising hyphæ to no great height above the surface. The advancing edge of the mycelium is white, but it shades a deeper and deeper green towards the centre, where the colour is a deep sage green. The green colour is given by a fine powder, and this powder is a mass of spores.

1. Mount a few spores in a drop of nutrient solution and hang in a damp chamber, following the directions given for mucor. Watch the germination of the spores.

The spores are spherical cells from 5μ to 6μ in diameter, with no obvious double wall and with granular protoplasmic contents. A nucleus has been observed in each. The conditions and course of germination are very similar to those of the spores of mucor. A germ-tube is protruded whose limiting membrane is continuous with the layer of the inner surface of the spore-wall. The tube increases rapidly in length and generally it attains some size before branching. As already described, on a suitable substratum, a dense mycelium of interlaced hyphæ is formed.

2. Take a small portion of the mycelium, tease it out well

with needles in a drop of water on a glass slide, cover with a cover-glass, and examine with a high power.

Note the branched hyphæ divided into rather long cells by cross division walls. All division walls are formed in the cell at the apex of each hypha, and the whole hypha therefore consists of an apical cell, and of cells successively cut off from it. Small vacuoles are to be seen in the copious granular protoplasm. Strasburger states that many small nuclei are present in each cell.

3. Treat with one per cent. solution of osmic acid.

The brown coloration shows abundance of fat. Fifty per cent. of the dry weight of old mycelium of penicillium is fat formed in the destructive metabolism of protoplasm. Iodine shows no starch, and iodine and sulphuric acid give no blue coloration with the cell-walls.

As the mycelium develops, very numerous branches rise erect in the air and branch copiously in a penicillate manner, forming a brush-like head. The erect branch is a **Gonidiophore** (or **Sporophore**), and its ultimate branches all reach to about the same level. At their extremities are club-shaped cells, **Basidia** or **Sterigmata**, from the swollen apex of each of which is formed, by a process of growth and constriction (**Acrogenous abjunction**) a series of **Spores** (**Gonidia**), the youngest and smallest of which is the one next the sterigma; the oldest, the one farthest off. The process of abjunction may continue for a considerable time if sufficient nutrient material is available. The abjointed gonidia form a string remaining attached to each other for some time. They are eventually set free by the dissolution of a connecting material. The sporophore makes no further growth after abjunction has once commenced. The spores are ready to germinate at once. They have a very wide choice as regards the nutrient substra-

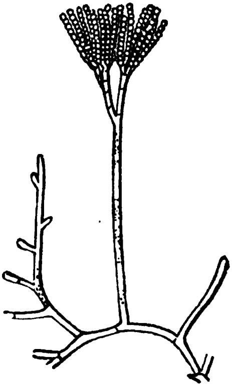

FIG. 30.—*Penicillum glaucum.*

tum on which they can germinate, and according to Wiesner a very wide range of temperature, the minimum being as low as 1·5° C. or 2° C., and the maximum 40° C. to 43° C. Like the spores of Mucor, the necessary and sufficient conditions for their germination are a supply of nutrient materials, water, oxygen, and some heat.

Other reproductive organs are produced by *Penicillium* when placed under conditions which do not often occur in nature. If the plant be allowed to make a vigorous growth under ordinary conditions, and be then placed in the dark with a good supply of nutrient materials, but a limited supply of oxygen, it forms no more gonidia, but produces **Sporocarps** by a process which is almost certainly sexual. Two short branches of the mycelium make one or two turns round each other in corkscrew fashion, closely applying themselves to each other, but no actual conjugation has ever been observed. One or both of these **Gametes** begins to branch, the branches eventually producing **Asci**, and at the same time neighbouring hyphæ grow up around them and finally enclose them in a many-layered cellular envelope, through the inner layers of which the **Ascogenous** hyphæ ramify. The sporocarp thus produced is spherical, yellow, and nearly half a millimetre in diameter. No further development takes place for seven or eight weeks, but at the end of that time, if the conditions are favourable, the ascogenous hyphæ in its interior produce asci, which are developed at the expense of the inner layers of the wall of the sporocarp. Each ascus contains eight ascospores. Finally the walls of the asci themselves are absorbed, and the sporocarp consists merely of a one-layered envelope enclosing a mass of **Ascospores** (see Fig. 31, which represents a process similar in the main to the development of the sporocarp of *Penicillium*). The ascospores germinate and produce a mycelium which bears ordinary sporophores.

The various species of *Penicillium* are saprophytes. Like the mucors, they can make use of ammonia compounds as well as of nitrates as sources of nitrogen, but they do best in a solution containing peptones and sugar. Again, like mucor, they can vegetate in the tissues of sound juicy fruit once the rind is broken for their entrance, and may so far be said to be parasitic, but no species of *Penicillium* is known to develop in animal bodies.

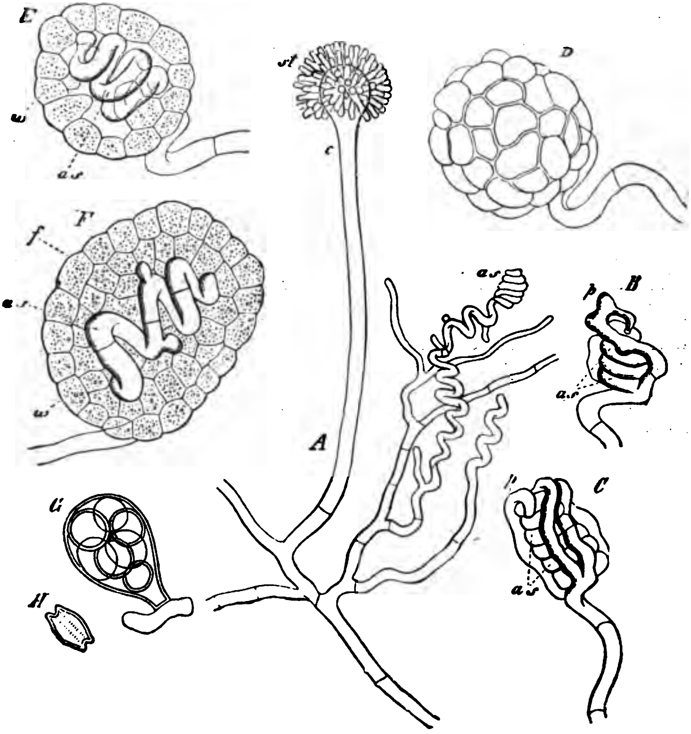

FIG. 31.—*Eurotium repens*. *A*, branch of mycelium; *c*, sporophore; *st*, sterigmata; *as*, carpogonium: *B*, *as*, spirally twisted carpogonium; *p*, antheridium; *C*, older stage with enveloping hyphæ; *D*, young sporocarp; *E*, *F*, young sporocarps in longitudinal section; *G*. ascus with spores; *H*, ascospore of *E. herbariorum*. (De Bary.)

The mycelium growing in or on the nutrient substratum is the absorbing portion of the plant, and the conditions of absorption are practically similar to those we had to deal with in the case of mucor, in spite of the fact that the hyphæ of Penicillium are septate, for each cell is directly in contact with its nutriment. But since the sub-aerial sporophores are also septate, we meet here with new conditions of distribution of the nutrient substances absorbed. The plant produces a continual series of gonidia, and there must therefore be a constant stream of plastic materials for the formation of the cell-walls and protoplasm of the spores ascending from the mycelium to the sterigmata and passing through the partitions which separate cell from cell of the sporophore. The passage through the partition walls is a process of osmosis of substances in solution, and the osmotic current is kept up so long as the plastic material is being removed from solution at the end of the sporophore (see p. 50), and this is as long as spores continue to be formed there.

Pasteur found that *Penicillium* also had the power of bringing about alcoholic fermentation in a sugared nutrient solution when submerged in it. Under these circumstances the cells of the hyphæ are larger in diameter and shorter in length than when growing in air, and sprouting takes place with the formation of gonidia. In addition some species of *Penicillium* produce an enzyme which converts cane-sugar into grape-sugar.

CHAPTER VI.

THE CHARACEÆ.

VARIOUS species of the Characeæ are universally spread—growing in sluggish rivers, lakes, and ponds; in fresh and brackish water at depths from a few inches up to six feet or more; or in swampy marshes, where they lie partly in and partly out of the water; but always necessarily wet. Rarely solitary, the plants usually form dense clumps, not unfrequently covering the bottoms of shallow lakes several acres in extent, to the complete exclusion of all other rooting plants. Their range of temperature is very wide, and one and the same species is capable of resisting the greatest extremes of heat and cold. Thus *Chara fragilis* is found everywhere—in this and other countries in water which in winter becomes a solid block of ice, and in the hot springs of the Yellowstone Park in the United States, where the water is "hot enough to cook an egg in four minutes."

The individuals of the different species are of all sizes, from a few inches up to three feet in height. From the lower portion of the stem, which is rarely more than one-twelfth of an inch, and generally much less in diameter, grow tufts of fine colourless threads, which root it in the mud at the bottom. The stem has growing from it whorls of green thread-like organs, usually called **Leaves,** which spring from it, five to fifteen at a level, arranged at equal angles around it. The lengthened portions of the stem are **Internodes**; the places at which the leaves arise are **Nodes.** Towards the summit of the plant the internodes and the leaves arising from the nodes are shorter, and at the apex all are crowded together in an **Apical Bud.** Similar buds may be seen tipping branches, having their origin in the nodes within the circle of leaves. The stem and leaves are green, the rootlets—or, properly speaking, **Rhizoids**—of which a great number arise from more than one node near the base of the stem, are long, thin, and colourless. Most species are encrusted with carbonate of lime, some so strongly as to assume a grey colour in consequence, and be easily broken when handled. Hence their common names of *Stone Worts* and *Brittle Worts* in this country. They are most of them very easily cultivated in very simple aquaria, and require no more attention than filling up with ordinary water as evaporation goes on. Some, however, require much more light than others.

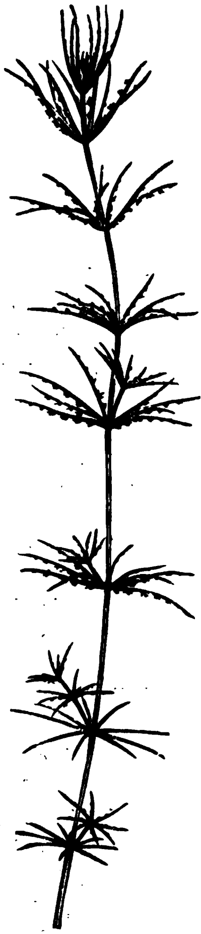

FIG. 32.—*Chara fragilis*. (Bennett and Murray.)

1. Examine with a hand-glass a plant lying in water. If much encrusted with lime, immerse it for some little time in weak vinegar and water to dissolve off the lime.

Note first the long internodes. In some species each consists of a single cell, in others this is **Corticated** by a layer of lengthened cells, which ascend from node to node in a steep spiral. Very frequently the corticated stem is beset with many long or short green **Spines**, and at the bases of the leaves, in the outer obtuse angle between leaf and stem, are often one, two, or even three circles of long or short green cells, which are turned, the upper circle up along the leaves, the lower down along the stem. They are commonly known as **Stipules**. The leaves also consist of nodes and internodes, and here also the internodes may be corticated or uncorticated. From the nodes of the leaf spring the **Leaflets**, which are generally longer on the side turned towards the stem—the **ventral side**—than on the other—the **dorsal side.** Note the crowded leaves and short internodes of the apical bud; note also that no internode attains its full size until some distance below the apex.

Borne on the nodes of the leaves of the upper whorls, near the bases of the leaflets at certain seasons of the year, are to be found two different kinds of **Sexual Organs**—the one, bright red and spherical, the male organ, called the **Antheridium**; the other, generally brown or black and oval in outline, the female organ, called the **Archegonium.**

2. Examine a ripe archegonium (fruit, or **Spermocarp**) in water under an inch objective.

The end may be crowned with a circle of five cells, or two circles of five cells each, or the **Crown** may have fallen off. Note the dark oval **Oospore** (egg) through the spiral corticating cells.

3. Press the cover-glass and burst the spermocarp so that the contents of the egg-cell are squeezed out. Examine with a higher power.

Note the numerous large granules. Run solution of iodine beneath the cover-glass. Large numbers of the granules take on a blue colour, showing that they are starch, and many small proteid granules are seen. Mount another spermocarp in solution of osmic acid and burst it. Many globules, some of large size, are turned brown. They are fat-globules.

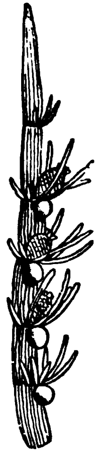

FIG. 33.—Fertile branch of *Chara hispida*. (Bennett and Murray.)

4. Prepare a small glass aquarium (a common pickle-bottle will do very well) by placing at the bottom an inch or so of washed garden mould or fine sand mixed with a little garden mould, and filling up with water. When clear, place in it some few plants of a common species of *Chara—fœtida* or *fragilis*—fruiting, *i.e.* with sexual organs on them. The spermocarps will drop off as they come to maturity and sink in the mud, but they do not germinate for some time. The safest

plan for getting a good supply of germinating spermocarps is to collect the fruiting plants in autumn and keep a good look-out for young plants early in the following spring. In the mud the corticating cells of the spermocarp rot off, leaving the oospore invested with their inner walls, ridges marking the former positions of the spirally disposed corticating cells. This investment may be called a **Pericarp** (Fig. 34).

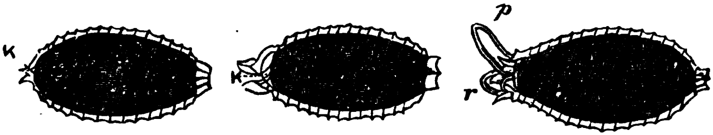

FIG. 34.—Spermocarp of *Chara fœtida.* K, the first node cell; *p*, primary shoot of the prothallium; *r*, primary rhizoid. (De Bary.)

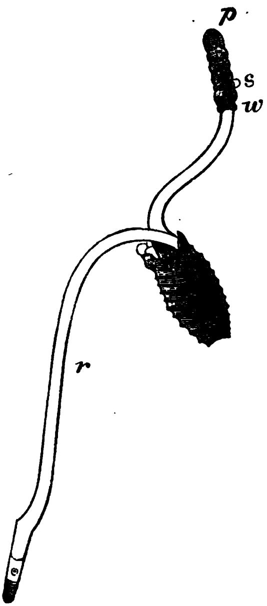

FIG. 35.—Germinating spermocarp of *Chara crinita.* *p*, apex of prothallium; *r*, rhizoid; *w*, root-node; *s*, stem-node. (After De Bary.)

The earlier stages of germination are difficult to make out. At the apex of the oospore the protoplasm is finely granular and translucent, whilst the rest contains the large starch and fat bodies, the two being separated by a clearly defined boundary plane. In this plane a cellulose wall appears, thus dividing the oospore into two unequally sized cells—the upper small one, the **First Node-cell** of the shape of a plano-convex lens, and the lower large one, the **Basal Cell** of De Bary. At the same time it swells somewhat and splits the pericarp at the apex along the spiral lines. The basal cell remains within the pericarp, and its reserve stuffs — starch, fat, and proteids—serve as a store of nourishment for the young plant; from the first node-cell, by repeated divisions and growth, are formed all the parts of the mature plant, and something besides.

The node-cell divides into two of equal size by a wall parallel to the axis of the spermocarp, and both these cells grow out into cylindrical tubes. The one, bending downward (Fig. 35, *r*), becomes the primary rhizoid of the **Prothallium** (**Pro-embryo**), the other is negatively geotropic (Fig. 35, *p*). It forms the primary shoot, with limited apical growth, of the prothallium, and divides by transverse walls into a small number of cells. Not unfrequently other, accessory, prothallia spring from the same primary node. From this stage the student should follow much of the development without difficulty.

5. Mount the prothallium in water, and examine.

No further division takes place in any of the row of cells of the prothallium except the lowest. Near the upper end of this lowest cell a transverse division wall appears, and the cell thus formed further divides into three cells, a middle rather long internodal cell, and an upper and a lower disc-shape cell (Fig. 35, *s* and *w*). The middle cell, as well as those above it, contains chlorophyll bodies. It develops no further, but the two disc-shaped cells are further modified. The lower cell forms division walls parallel to the axis of the prothallium until it becomes a cell-layer whose peripheral cells grow out into rhizoids. It thus becomes the **Root-node** of the prothallium. The upper cell divides somewhat similarly and forms the only **Stem-node** of the prothallium. *h* (Fig. 36) is the first division wall which halves it. Then other walls are formed, which cut off in succession, right and left of *h*, the cells *i*, *b*, 3, 4, 5, 6, 7. *b* and the succeeding peripheral cells develop into leaves, which only differ from the portion of the prothallium above the node in origin and size. They never develop lateral leaflets, nor sexual organs, and are of definitely limited apical growth. The first-formed peripheral cell, *i*, is the apical cell of a sprout of unlimited apical growth, the ordinary chara

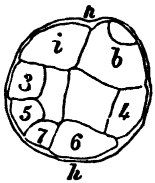

FIG. 36.—Transverse section through the stem-node of a prothallium of *Tolypella intricata*. *i*, initial cell of the first stem; *b*, of the first leaf. (De Bary.)

plant, which, as we thus see, has a lateral origin in the prothallium. Thus the spermocarp always gives rise to a prothallium, which always produces a chara plant by a lateral bud, which again always produces spermocarps, although it may bring forth a prothallium directly without the intervention of a spermocarp. Thus the chara plant is a **Gametophyte** (bearing several organs), which can in some species be **Apogamous.** It is perhaps right not to speak of an alternation of generations in this connection, but only of an alternation of axes.

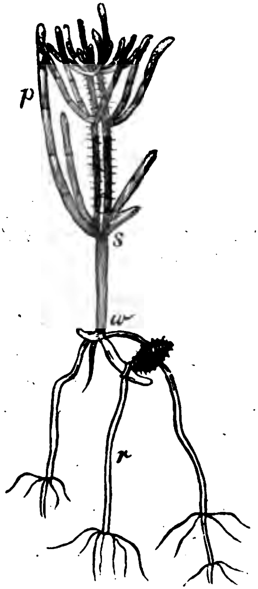

FIG. 37.—Young plant of *Chara crinita.* Letters as before. (After De Bary.)

Generally only one gametophyte originates in the prothallium, rarely two or three.

6. Cut off the apical portion of a stem of Nitella, place it in a little water under a simple microscope, and with a couple of needles dissect away as many of the more developed whorls of leaves as possible without injury to the actual apex. If Nitella is not available, *Chara fragilis* will serve. Fresh material will do very well, but plants fixed in chromic or picric acid, as afterwards described, are better. Those in which the nucleus has been stained are the best of all. Place the actual apex in water on a slide, cover, and press steadily. If two or three are thus prepared, one will certainly be suitable for the examination of the apical cell and its segments. Examine.

The growth of the characeæ is acrogenous by means of an **Apical Cell** of the form of a plano-convex lens, from the base of which cells are cut off in succession, the end segment remaining an apical cell with unlimited power of producing segments (Fig. 38). Each cell cut off from the apical cell divides again immediately by a transverse wall into an upper larger cell—a nodal cell—and a lower smaller cell—an internodal cell. The internodal cell never divides, but increases very considerably in size; the nodal cell increases much less in size, but divides by partition walls, mainly in vertical planes, as already described in the stem-node of the prothallium (Fig. 36), forming in all cases a cell-layer of peripheral and two central cells. The internodal cell, corticated or uncorticated, forms an internode of the plant. It attains a great length, reaching in the subterranean branches of *Lychnothamnus stelliger* even eighteen inches or more. From the peripheral cells of the node are developed leaves, one from each cell, the number in each whorl being equal therefore to the number of peripheral cells in the node, and varying from five to fifteen. The leaves of the separate whorls do not stand vertically over each other, but alternate one with another, so that each leaf

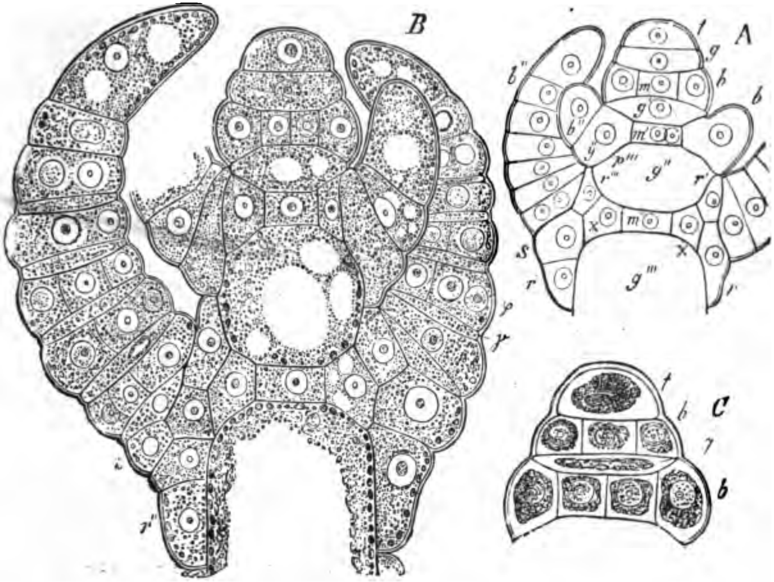

FIG. 38.—Longitudinal section through the bud of *Chara fragilis*, showing apical cell (*t*), and its segments (*g* and *b*). *A*, with empty cells; *B*, cells with contents; *C*, cell-contents contracted by iodine. (Sachs.)

bisects the angle formed by two adjacent leaves in each of the whorls immediately above and beneath it. The divergence from a vertical line in the arrangement of the leaves depends therefore on the number of leaves in a whorl. If there are six leaves the divergence is an angle of $\frac{1}{2}$ of $\frac{360^\circ}{6}$; if seven, an angle of $\frac{1}{2}$ of $\frac{360^\circ}{7}$, and so on. The usual mode of expressing this is by putting it as a fraction of the whole circumference, and saying that the divergence is $\frac{1}{12}$, $\frac{1}{14}$, etc.

7. Detach a leaf, and examine with a low power.

The leaves are of limited growth, but show otherwise in their development some similarity to the stem. Nodal and internodal cells are produced and the latter may be all uncorticated, or most of them may be corticated. In all cases, however, the end cell, and generally some others beneath it, are naked. The nodal cells divide as other nodal cells do, and from their peripheral cells the lateral leaflets and other structures are developed. These lateral leaflets are rarely more than one-celled. They are seldom equally developed all round, but usually more so on the ventral than on the dorsal side, the latter frequently appearing as mere warts (Fig. 33).

The first cell cut off from the mother-cell of the leaf divides into an upper **Nodal Cell**, and a lower cell (*Verbindungszelle*), which corresponds to an internodal cell. This latter does not lengthen as other internodal cells do, but serves to connect the leaf with the central cell of the stem node (Fig. 38, A y''). The nodal cell develops into a disc of cells, which is known as the **Basal Node of the leaf.** This basal node is the origin of very different and peculiar organs.

There arise from it, *first*, the so-called **Stipules**, when any are present. These are always one-celled, in some species long, in others wart-shaped, embracing the

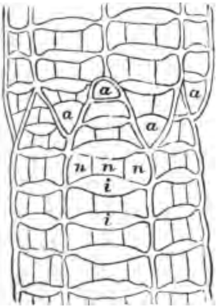

FIG. 39.—Development of the cortex of the stem of *Chara fragilis*. *a*, apical cells of corticating lobes; *n*, nodal cells; *i*, internodal cells. (After Sachs.)

stem at the base of the whorl of leaves in one, two, or even three circles.

Secondly, the cortication of the stem has its origin in the basal node of the leaf, every two consecutive whorls contributing to the cortication of the internode between them. From the basal node of every leaf grow two corticating mother-cells in the form of rounded lobes, and proceed in opposite directions, one upwards, the other downwards. They appear early, before the internodal cell of the stem has lengthened much, and the growth of the corticating cells keeps pace with its growth, so that the internodal cells of the stem are, in corticated species, naked at no period of their development. The apical cells of the descending and ascending corticating series alternate with each other, and consequently where they meet they dovetail with each other (Fig. 39). This must follow from the fact that the leaves of successive whorls alternate with each other.

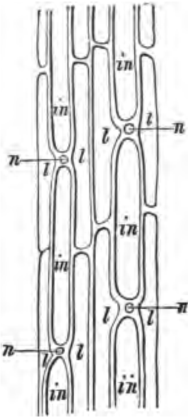

Fig. 40.—Cortex of *Chara fragilis*. *n*, the nodal, *in*, the internodal cells of the primary corticating series; *l* are the lateral cells developed from *n*, and which triply corticate the stem. (After Allen.)

Each corticating series consists of alternate long internodal and short nodal cells. These nodal cells may develop in various ways, and double or triple the corticating rows, as in Fig. 40, or they may grow out into unicellular spines or hairs, or may give rise to both. As examples, *Chara crinita* has as many corticating rows as there are leaves in a whorl, *Ch. fœtida* has twice as many, and *Ch. fragilis* has three times as many. Again, the corticating nodal cells produce tufts of hairs in *Ch. crinita*, warts in *Ch. fœtida*, and neither hairs nor warts in *Ch. fragilis*.

Thirdly, every leaf-whorl possesses the power of developing a branch from the basal leaf-node of the first formed leaf of the whorl. It frequently does not develop much. It stands, in chara, in the *axil* of the leaf; in nitella, somewhat laterally, and here also a second smaller branch may sometimes be found. These normal branches are similar in every respect to the main axis. There are, however, very few, or probably no species of characeæ which cannot later develop other branches from its nodes, and these are always abnormal in structure in some respect.

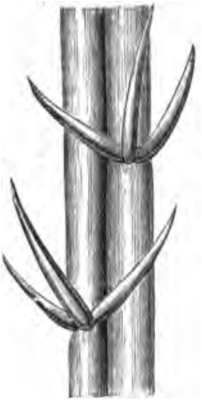
FIG. 41.—Cortex of *Chara crinita*, showing tufts of hairs developed from corticating nodal cells. (Allen.)

The cortication of the leaf is a process similar to that of the stem, but simpler. The corticating cells are developed out of the basal nodes of the leaflets.

The sexual organs of the characeæ are of two kinds, **Antheridia** and **Archegonia**. They are always developed on the leaves of the plant. In some species both organs are borne on the same node or on different nodes of the same plant, when the plant is said to be **Monœcious**; in others, the male organs are borne on one plant and the female on another, and then the plant is called **Diœcious**.

The male organ, the antheridium, always either terminates a leaf or occupies the position of a leaflet, being in that case developed from just such a cell as that from which a leaflet is formed. The female organ, the archegonium, is developed from a cell of the basal node of a leaflet, or of the antheridium developed in the place of a leaflet. The antheridia vary in diameter in the different

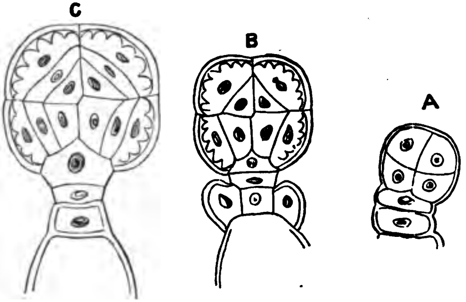

FIG. 42.—Development of the antheridium of *Nitella flexilis*. A, B, C, successive stages of development. (After Sachs.)

species from ·5 up to ·8 millimetre, the archegonia in length from ·35 up to 1·25 millimetre.

8. Select an apical bud, bearing sexual organs, of *Chara fragilis* or some other species, dissect it out in a drop of water on a slide, cover, and examine for developing sexual organs on the youngest leaves. Do the same with chromic acid or picric acid material, and examine in glycerine.

The course of development of an antheridium is shortly this. The mother-cell, resting on its basal node, divides into an upper hemispherical and a lower disc-shaped cell. The lower forms a short stalk; the upper, by successive walls in planes perpendicular to each other, divides into eight cells, the octants of a sphere, the antheridium becoming all the while more and more spherical. Of the eight cells meeting in the centre, four are above and four below, or four belong to the right side and four to the left. Walls are now formed parallel to the surface which divide the eight cells into

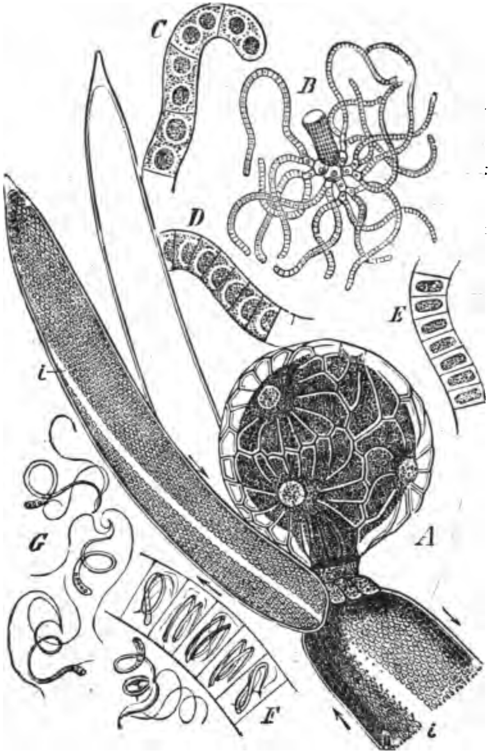

FIG. 43.—*Nitella flexilis*. *A*, nearly ripe antheridium; *B*, manubrium, with capitulum, secondary capitula, and filaments; *C–F*, filaments showing formation of antherozooids; *G*, antherozooids. (Sachs.)

sixteen, eight outer and eight inner, and the latter divide again immediately in such a way that the whole antheridial sphere now consists of eight outer, eight middle, and eight inner cells —three layers of eight cells each. The outer cells are arched plates, the four upper of the form of equilateral triangles, the four lower triangles truncated at the angles in contact with the basal cell. The outer surfaces of these plates become infolded, from the centre outwards, in rayed pleats which reach the opposite inner wall of the cell (Figs. 42, 43), and which cause the pretty rayed appearance from the surface (Fig. 43, *A*). The cells of the middle layer grow only radially, and to a slight extent. Their outer ends remain connected with the centres of the triangular peripheral cells, whilst their inner ends are joined with the central cells. The peripheral cells outstrip the cells of the middle layer so completely in their rate of growth that large intercellular spaces are formed between the latter. These cells of the middle layer are called **Manubria** (handles). The innermost eight cells are spherical **Capitula** (heads), which are in contact with each other at the centre of the sphere. Each capitulum sprouts and gives rise to from four to six small round cells, from every one of which again from three to five still smaller cells grow, which develop later into long, thin, backward-growing, many-celled filaments, which fill up the spaces between the manubria (Fig. 43, *B*). Each cell of this thread shows a large nucleus, out of which a very fine, spirally-twisted **Antherozoid** is formed, with a blunt posterior, and a sharp anterior end furnished with two vibratile cilia (Fig. 43, *G*). The cilia are formed out of the general protoplasm of the cell.

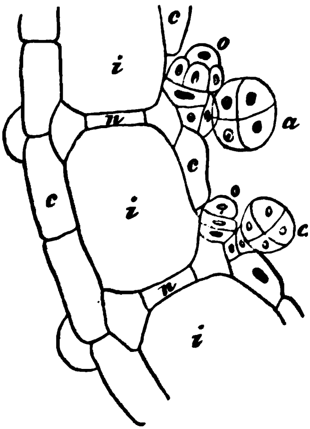

FIG. 44.—Portion of a fertile leaf of *Chara fragilis* with developing sexual organs. *a*, antheridia; *o*, archegonia; *i*, internodal cells; *n*, nodal cells; *c*, corticating cells. (After Sachs.)

The mother-cell of the antheridium contains chlorophyll corpuscles, and so do later the peripheral cells and manubria. They become changed, however, to red corpuscles, which in the outer cells lie close to their inner walls, giving the antheridium an appearance as if surrounded by a deep, colourless, hyaline border (Fig. 43, *A*). Frequently the antheridium has in addition a coating of mucilage.

9. Mount a leaf bearing antheridia and archegonia in water, and examine with a low power.

Note the attachment of the antheridium to the inner (ventral) side of the leaf at the node, lying below the leaflets, here frequently called **Bracts.** By the growth of the archegonium above it and of the bracts, it is pushed into a lower position than it would otherwise occupy (Fig. 46). Note the outlines of the peripheral cells and the rayed appearance of the surface.

10. Detach an antheridium, mount it alone in water, and crush it by pressure on the cover-glass. Examine with a higher power.

Note the manubria and capitula. Mark the filaments, their constituent cells, and their very evident nuclei. The incomplete divisions near the tips of the filaments show that their growth is **apical.** Also look further back for divided nuclei, between which no division wall is yet formed. Many instances of such will be found, and the growth of the filament is therefore **intercalary** as well as apical. In ripe antheridia, cells of the filaments will be found containing partially or completely formed antherozoids.

11. Tease out an antheridium on a slide in a drop of water, add a trace of gentian violet, cover with a cover-glass, and then apply a piece of blotting-paper at one side, whilst running in pure water at the other. In this way minute objects can be stained and washed, which by other methods could not be safely handled. Examine with a high power.

The nuclei of the filament cells, and the antherozoids where any are developed, are now very evident. Note also the large oval nucleus in some manubria, or more than one irregularly shaped nucleus in others.

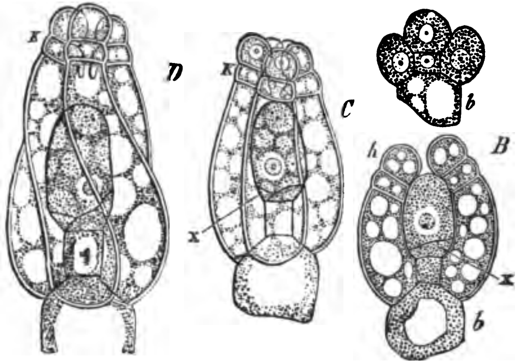

FIG. 45.—Development of the archegonium of *Nitella flexilis*. *x*, wendungszellen; *k*, crown; *b*, summit of fertile leaf. (Sachs.)

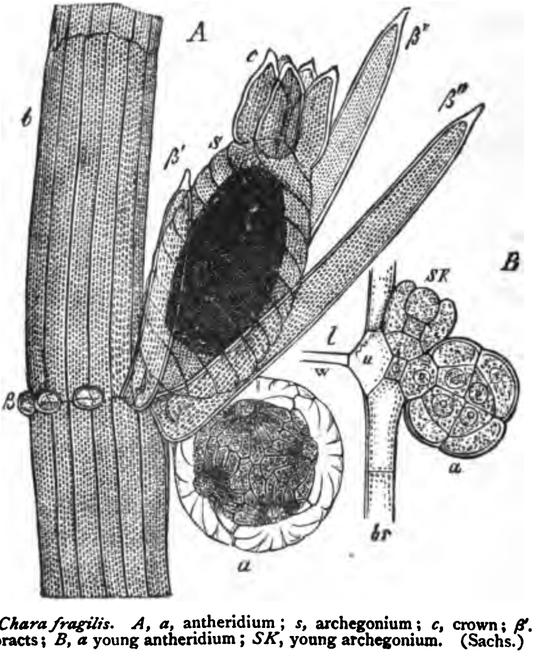

FIG. 46.—*Chara fragilis*. *A*, *a*, antheridium; *s*, archegonium; *c*, crown; β'. β'', β''', bracts; *B*, *a* young antheridium; *SK*, young archegonium. (Sachs.)

12. Mount leaves bearing archegonia in various stages of development, and follow as far as possible.

The mother-cell of the archegonium divides by transverse walls into an axile row of three cells, the lowest of which is a nodal cell, and the uppermost the **Oosphere**, or egg-cell (Fig. 44). From the nodal cell are separated five peripheral cells, which grow out into corticating tubes and closely invest the oosphere. At first they are straight, but later they become twisted into a right-handed spiral. Each corticating tube divides once in charæ and twice in nitellæ, making in the former a single, and in the latter a double **Crown**, which overtops the oosphere.

The oosphere completely fills the envelope. It is at first filled with homogeneous protoplasm, from which starch-grains and fat-corpuscles separate. At the apex is finely granulated protoplasm. The cell-wall of the oosphere is extremely delicate. At the apex it softens to a jelly or even disappears entirely, the cells of the crown forming a covering for it there.

The ripening of the oosphere takes place usually at the same time as the breaking up of the antheridium and the consequent freeing of the antherozoids. Then the corticating cells suddenly lengthen in the region close to the lower part of the crown and develop a neck, but the separate neck-pieces do not close completely together. There are thus narrow fissures between them, as well as a large free inner space under the crown and over the top of the oosphere. The antherozoids, moving by the aid of their cilia, pass through the fissures in the neck into the space under the crown and thence to the apex of the oosphere, which they no doubt enter and fertilize. The oosphere is now an **Oospore**, capable of reproducing a plant similar to its parent plant.

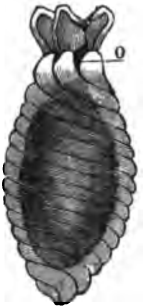

FIG. 47.—Archegonium of *Chara intermedia* ready for fertilization. *o*, opening beneath the crown to admit antherozoids.

Chara crinita, a diœcious corticated species, in which the rows of corticating cells are equal in number to the leaves in each whorl, rarely produces male plants bearing antheridia, although female plants bearing archegonia are frequently met with. Nevertheless, in circumstances under which fertilization is impossible, *e.g.* when female plants are completely isolated, their archegonia are capable of germination. Such cases of **Parthenogenesis** are uncommon, although known to occur in other members of the vegetable and animal worlds.

In none of the characeæ has **asexual** reproduction by means of spores been observed. There are, however, amongst most, and probably all species methods of **vegetative** reproduction which are very efficacious in continuing the life of the individual. These are, by means of abnormal **adventitious** branches, from stem and root nodes. The first branch from a stem node is a normal axis similar in all respects to the parent axis, but under certain circumstances other branches may arise from the same node, and these adventitious branches are always abnormal. In *Chara hispida*, *Ch. fragilis*, *Ch. fœtida*, *Ch. aspera*, *Lychnothamnus stelliger*, *Nitella opaca*, and others, the cells of the basal leaf-nodes, which arise from the older stem-nodes, divide and subdivide until the stem-node is a swollen more or less star-shaped mass of cells, which are crowded with starch-grains and other plastic substances. The internodal portions of the plant decay and the isolated swollen nodes sink into the mud. Later, certain cells grow out into branches, which are so far abnormal in structure that the lowest internode, and in many cases the second and third, as well as the leaves of one or more whorls, are naked in the corticated species. These **Naked-footed (gymnopodal)** branches are not unfrequently produced from the nodes of normal axes, which have sustained injury. The branches which arise from the wintering nodes of uncorticated species (*Lychnothamnus*, *Nitella*, etc.) are always abnormal in the development of the leaves of the first few nodes. *Lychnothamnus stelliger* produces subterranean branches devoid of chlorophyll, which run horizontally through the mud and bear beautifully regular, stellate, swollen nodes

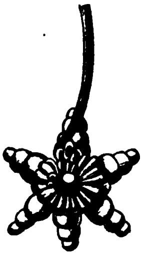

FIG. 48. — Starch-star of *Lychnothamnus stelliger* producing a branch.

on long transparent internodal cells, some of which are as much as eighteen inches in length.

The branches arising from root-nodes are also gymnopodal. Rarely such branches spring from ordinary root-nodes; one or more from a root-node of *Chara aspera* are not very uncommon. Generally one or a few cells of the root-node increase enormously in size, become distended with plastic materials, and form, with the other undistended cells of the node, a **Wintering Bulb** which is capable of producing a new plant. This is the general case with *Chara aspera.* When such a bulb germinates it produces a gymnopodal structure which is similar either to the prothallium formed from the spermocarp (Fig. 49) or to the gymnopodal branches formed from old nodes. Pringsheim describes similar **Prothalloid Branches** as arising from old nodes of *Chara fragilis.*

By one or more of these vegetative methods of reproduction the life of the individual plant may be prolonged for several years. Such plants are said to be **Perennial.**

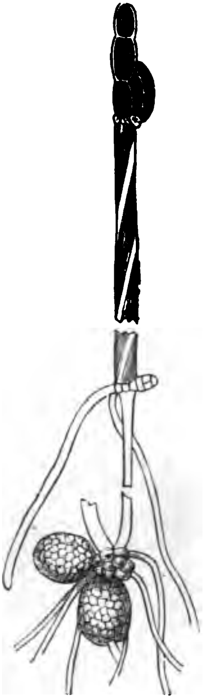

FIG. 49.—Starch-bulbils of *Chara aspera* producing a prothalloid branch.

13. Test the cell-wall of nitella or chara with iodine and sulphuric acid, or with Schulze's fluid.

The cell-wall is coloured blue, showing that it is composed of cellulose.

No differentiation of the cell-wall into layers can be observed, but that there is a **middle layer** of different constitution between adjacent cell-walls, is shown by the following :—

14. Boil any species of characeæ for half a minute in dilute caustic soda. Select an apical portion or rhizoids, and mount in water. Cover, press the cover-glass somewhat, and examine.

The pressure separates adjacent cells from each other, the cells remaining intact. This can only come about by the solution of a layer of substance lying between adjacent cell-walls. By this method also especially clear apical portions can be prepared. The preparation stains well with aniline colours, and its value for study is thereby increased.

The Cell Contents.

15. Mount a leaf of nitella in water, and examine with a low power.

Focus the surface of the leaf and notice the light-green chlorophyll bodies lying immediately beneath the cell-wall. They are especially large, distinct, and well separated from each other in the lower part of a young leaf. They vary slightly in shape, but are usually more or less elliptic in outline. Their longest diameter is from 8μ to 10μ. Note that they are arranged fairly regularly in spiral lines running the length of the leaf. Two clear lines, devoid of chlorophyll bodies, also run the length of the cell in a steep spiral. These are the **Indifferent Lines**. The outlines of contained starch-granules are very evident in the greater part of the corpuscles when a higher power is used.

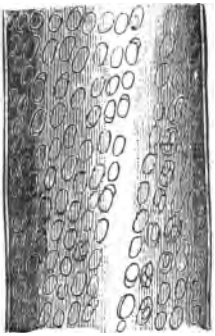

FIG. 50.—Portion of leaf of *Nitella flexilis*, showing chlorophyll bodies and the indifferent line.

16. Lay the upper portion of a nitella plant in weak solution of iodine in potassium iodide for a few minutes. Wash and mount a leaf in water. Examine with a high power.

The starch-grains in the chlorophyll bodies are stained blue, and are now easily distinguished, although the simultaneous yellow coloration of the proteid matter of the bodies, together with the green chlorophyll, obscures the colour somewhat. Some contain many starch-grains, others but one, so large that it nearly fills the corpuscle. If a leaf be laid in alcohol until the chlorophyll is dissolved out, and then in solution of iodine, the starch-grains in the chlorophyll bodies are well seen.

17. Examine the upper portion of a growing leaf of nitella in water with a high power.

Look for chlorophyll bodies which are in the various stages of dividing by constriction. They are very numerous.

All these points can be made out in the cleaner of the corticated species of chara, but not so well as in nitella.

18. Mount a leaf of nitella in water, and focus below the surface-layer of protoplasm containing the chlorophyll bodies with a moderately high power.

The protoplasm beneath the peripheral layer is in motion, forming a current which ascends one side of the cell and descends the other, the lines of separation being the indifferent lines. Many solid bodies are carried along in the stream, and by watching them it is easy to see that the protoplasm in contact with the motionless peripheral layer moves the fastest, and that the watery cell-sap in the centre of the cell is also motionless. Since the indifferent lines are spirals, it is evident that the currents also follow spiral courses. In all cells except the youngest, some nodal cells, and some cells of the sexual organs, this **Rotation** of the protoplasm goes on.

In corticated species the rotation can be studied in the end-segments of leaves, and in the cells which corticate the young oosphere. The best cells for the purpose are the rhizoids of all species, the colourless lower internodes of gymnopodal branches and prothallia, and internodes of the subterranean branches of *Lychnothamnus stelliger*. In these latter, and in larger rhizoids, the thickness of the rotating layer of protoplasm is seen to be equal to about one-fourth the diameter of the cell, its inner surface being very sharply separated from the watery cell-sap.

19. Press out the contents of an internodal cell of nitella into a drop of water on a slide. Cover, and examine with a high power.

The expressed protoplasm is at first a viscid mass which is coherent enough to be lifted out of the water on the point of a needle. It slowly mixes with the water and its more solid constituents float free. The proteid matter of the chlorophyll bodies is disorganized with the water, and their contained starch-grains swell somewhat. The large rounded balls which were seen floating in the protoplasmic stream of the uninjured cell are isolated first, and float freely, showing that the protoplasm which contained them was more easily miscible with water than that which contained the chlorophyll bodies. Note also an immense number of minute granules scattered throughout the protoplasm. They are also of a proteid nature, and are called **Microsomata.**

20. Run solution of iodine under the cover-glass.

The starch-grains now show the violet colour well. The rounded bodies show their proteid nature by taking on a yellow colour.

The starch-grains of different species of characeæ, formed under similar conditions, vary slightly in shape and size. Those formed in chlorophyll bodies are always more or less elliptical in outline and lenticular in form. When formed, however, in parts of the plant devoid of chlorophyll, such as bulbs, swollen nodes, and oospheres, some of them are of much larger size, of more rounded form, and show a faint indication of lamination.

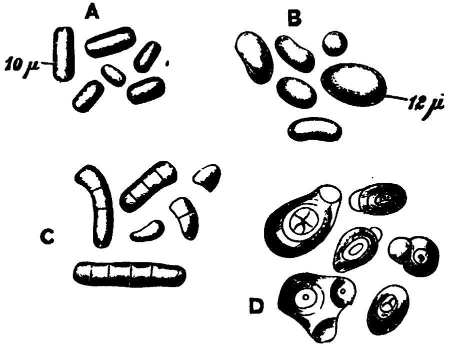

FIG. 51.—Starch-grains of *Characeæ*. A, from leaf of *Chara fœtida*; B, from leaf of *Ch. hispidia*; C, compound grains from an old internode of *Ch. fragilis*; D, from starch-star of *Lychnothamnus stelliger*.

Starch-grains obtained from different plants vary greatly in size and shape, although all agree in this, that they are never angular, but their surfaces are always rounded. Compound grains are known, however, which break up into angular fragments. Many starch-grains are obviously laminated, consisting of less dense and denser layers deposited around a point in the grain called the **Hilum.** This lamination is not visible in some grains. In polarized light each grain appears with a dark cross on a light ground, and this shows that the tension in the grain is greater in one direction than in the other. The cohesion of the particles in the grain is not great. If some grains be mounted in water, and pressure applied to the cover-glass, the circumference, instead of being stretched, gives way, and radial clefts appear, as they do in a half-dried pellet of clay when subjected to pressure.

So far as is known all starch-grains are formed in the interior of proteid bodies called **Plastids (Amyloplasts).** These are of two kinds, **Chloroplasts** (chlorophyll bodies), or green starch-formers, and **Leukoplasts,** or colourless starch-formers, and they are produced by the actual conversion of a portion of the protoplasm of the amyloplast into starch, each layer being deposited on the outside of the grain. When starch is rubbed up with fine sand in water, the friction with the sand breaks the grains and a small portion is dissolved. This soluble starch is called **Granulose,** the insoluble portion **Starch-cellulose.** Treated with hot water the grains swell up enormously and burst—a small portion dissolving, and the whole forming a paste which when cold congeals into a jelly. Boiled with a large quantity of water it filters slowly and badly; allowed to stand, a large portion settles at the bottom of the vessel, the supernatant liquid being fairly clear. This latter contains starch in solution, although always much starch-cellulose in suspension. The longer the starch is boiled the more it enters into solution, although it appears that some portions of the grain never dissolve in pure boiling water. Addition of alcohol to starch paste causes agglomeration and deposition of the starch. A solution of potash causes starch-grains to swell up enormously, and to become so nearly equal in refractive power to water that they are scarcely visible in that liquid under the microscope.

Starch is manufactured for commercial purposes by mechanically bursting the cells containing the grains, thereby freeing them, and afterwards washing them out by water. A paste made from a sample of such starch, showing under the microscope nothing but starch-grains, will nevertheless nourish such fungi as *Mucor* and *Penicillium*, showing that there is nitrogenous organic matter in the grains, although the amount must be slight.

With dry starch-grains free iodine gives a black colour, but in the presence of water the colour is a deep blue. Only the granulose of the grain is coloured blue, the starch-cellulose reacting as other cellulose does. The blue substance is known as iodide of starch, but is not a true compound. Boiling with a mineral acid converts starch first into dextrine and finally into grape sugar; and the same conversion into grape sugar is brought about by various enzymes. Thus, if saliva be mixed with starch paste, and the mixture kept at about 100° F. for a few hours, iodine will no longer give a blue colour with it, but the substance will reduce Fehling's solution readily, showing the disappearance of starch and the production of grape sugar. The secretion of the pancreas of animals brings about the same result. Many such enzymes are found in plants, the most familiar example being the **diastase** of germinating seeds. In all parts of plants where starch is formed or stored, enzymes capable of converting starch into sugar are to be found. Starch is not diffusible, and therefore cannot pass through closed cell-walls from one part of the plant to another. Grape sugar is diffusible, and in this form the transport of carbohydrate plastic material takes place. In the germinating starch-stars and bulbs of the characeæ grains of starch partially dissolved are commonly to be seen.

The proteid balls in species of nitella are spherical bodies covered all over with small spines. In chara they are smooth, more or less irregular in shape, homogeneous and highly refractive. They can be readily studied in the protoplasm squeezed out of an internodal cell, especially in the winter.

In such cells also other bodies are visible which ordinarily require special methods of fixing and staining to recognize them. These are **Nuclei**, of which there are many in each internodal cell. Some are straight cylinders with rounded ends, others are slightly curved, and others still are bent nearly into the shape of a horseshoe. In a plant in active growth the density of the nuclei is much less, and they are rapidly broken up when subjected to the action of water. For the same reason they cannot be distinguished in the rotating stream of protoplasm in the living cell. If the contents of an internodal cell be pressed out into a drop of a weak solution of gentian violet, the nuclei are well stained. In them will be seen irregularly shaped particles which stain more deeply than the rest. These are **Nucleoli**, of which there are several in each nucleus.

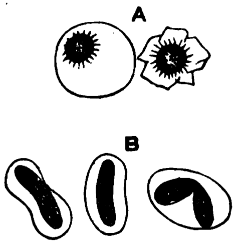

FIG. 52.—A, spiny proteid balls of *Nitella flexilis*; B, nuclei of internodal cell of the same plant; both in water.

21. Study the contents of the cell by the following method:—Place some young shoots and rhizoids of a species of characeæ, preferably a nitella, in a concentrated solution of picric acid, and allow to remain at least twenty-four hours. Then wash frequently in large volumes of distilled water until all trace of picric acid is washed out of the plant. Make a solution of hæmatoxylin by placing a piece about the size of a grain of wheat in about 20 c.c. of distilled water, and stirring with a glass rod whose point has been dipped in solution of ammonia. The smallest trace of ammonia brings about the solution of the hæmatoxylin. Add enough distilled water to make up to 50 c.c., and in this place the well-washed nitella. It may remain there with advantage up to twelve hours. Then again wash in water, mount a portion, and examine.

The picric acid fixes the contents of the cells in their natural positions. Nothing is stained but the nuclei, which take a violet colour; the nucleoli a deeper shade of violet.

22. Examine the nuclei of various cells from the apical cell downward.

The apical cell, and all others which retain the power of division, have but a single nucleus containing a large nucleolus. The division of such a cell is preceded and brought about by the division of its nucleus, the new cell-wall being formed between the daughter-nuclei. The stages of the division of the nucleus, which are not easy to make out, appear to be as follow: The nuclear substance breaks up into a number of particles, which arrange themselves equatorially in the plane of the future cell-wall. This is the **Nuclear Plate,** and from its particles proceed fine filaments whose distal extremities meet in the two poles. These filaments are **Spindle-fibres,** and the whole of the nucleus in this stage is known as a **Nuclear Spindle.** Next, the coarse chromatin particles of the nuclear plate separate from each other and retreat to the two poles of the nucleus, being connected by fine threads which are known as **Connecting Threads,** and in the equatorial plane appear one or two layers of particles which are larger than the microsomata of the surrounding cytoplasm of the cell, and which are swellings of the connecting threads. This is the **Cell-plate,** which as a rule touches the outer wall of the cell in one place only. When this is the case a spreading of the connecting threads follows, until the cell-plate fills the whole cross-section of the cell. Very rapidly the cell-plate is converted into a plate of cellulose, and the division of the cell is complete. The sister-nuclei now round themselves off and retreat from the new cell-wall, and the chromatin particles collect together again. The whole process is known as **Indirect Nuclear Division,** and division of the nucleus is of this character in all cells which retain the power of division. Such cells are the apical cells and their primary segments which divide into nodal and internodal cells, the cells of the prothallium, of the leaves and of the corticating lobes up to a certain stage, and the cells of the nodes: also the basal node-cells of leaves which may subsequently produce gymnopodal branches, the cells of secondary leaflets from which are produced the sexual organs, and the end cells

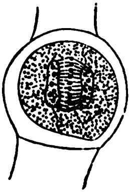

FIG. 53.—Division of the nucleus in a cell of a root-node of *Chara fœtida.* Stage showing cell-plate and connecting threads. (Johow.)

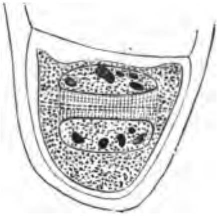

FIG. 54.—Nuclear division in end cell of a leaf of *Chara fœtida* showing newly formed cell-wall. (Johow.)

of rhizoids which form root-nodes. The last-named cells, however, show some features in their division different from the others. In the rhizoids, division of the nucleus and of the cell takes place after the cell has advanced so far that its centre is occupied by a large vacuole and its protoplasm is in active rotation. The end of such a cell is occupied by a mass of resting protoplasm, in which the nucleus lies.

But in all those cells which have not the power of division the nucleus breaks up by simple constriction of its envelope and separation of its parts into a large number of fragments which are distributed throughout the cell. This process is called by some **Direct Nuclear Division,** by others **Fragmentation of the nucleus.** Nuclei in all stages of division by constriction can be seen in the actively growing internodal cells near the apex of the stem. There is little doubt that the nuclear fragments are concerned in the nutrition of the very elongated cells.

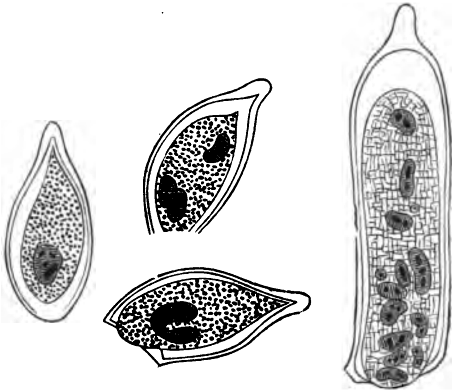

FIG. 55.—Successive stages of fragmentation of the nucleus in leaves of *Nitella flexilis*.

23. Press out into a drop of water the contents of an internodal cell of nitella which has been treated as in 21.

The motionless layer of protoplasm containing the chlorophyll bodies is coagulated into a coherent layer retaining the chlorophyll bodies in their natural positions. The proteid balls and the nuclei are not imbedded in this layer, but float free in the water, showing that in life they were contained in the internal more fluid portion of the protoplasm which has not been coagulated by the action of the picric acid.

All said about the conditions of life and growth and course of constructive and destructive metabolism in connection with protococcus applies with equal force to the characeæ. The plant receives its food from the water which is its environment, although we must here distinguish between those parts of the plant which are green and can therefore decompose carbon dioxide, and those parts which contain no chlorophyll and are therefore dependent on the green assimilating organs. The non-green portions, such as the rhizoids, stand, to the green organs somewhat in the relation of a parasite to its host. All nutrient matters pass from the stem to the rhizoids in solution through closed cell-walls. It is doubtful whether the rhizoids exercise any other function than the mechanical one of holding the plant in position.

The presence of reservoirs of **Reserve substances**, such as bulbs and swollen nodes, is also a distinction. Starch and proteids which are formed in the green assimilating organs are brought into solution and then diffuse to the growing point, where they are being used to form new cells, and to the reservoirs, where they are being stored up. The formation of starch-grains in such parts is not a physical one of mere agglomeration, but organized proteid particles are present which take up the plastic materials and form starch-grains in a somewhat similar manner to their formation in chlorophyll bodies. These **Starch-formers** are difficult to demonstrate in chara; they are known as **Leukoplasts**.

The passage of plastic substances from cell to cell is much facilitated by the rotation of the protoplasm in the elongated cells. The rotation is independent of light. It goes on as well in the rhizoids buried in the mud as in the internodal cells exposed to light—at night as well as by day. A temperature near freezing causes it to cease, but the most delicate of the species, *e.g. Nitella flexilis*, may be frozen in a solid block of ice, and the rotation recommences when slowly thawed. A high temperature coagulates the protoplasm, and thus causes the rotation to cease, yet, as we have seen, the plant may be inured to very high temperatures. The energy necessary for the rotation of the protoplasm is obtained from the oxidation which goes on in the cell. In an atmosphere deprived of oxygen the movement ceases. Thus Schnetzler, experimenting with the prothallium of *Chara fragilis*, found that there was no rotation in hydrogen or nitrogen, or when the cell was immersed in olive oil.

The nature and function of the rounded proteid balls which are found in the protoplasm are unknown. They are apparently quite inert, and are probably formed from the cytoplasm of the cell by a process of agglomeration.

If the plant, in water, be placed in direct sunlight, the absorption of carbon dioxide and production of oxygen is so vigorous that the whole plant becomes covered with bubbles of gas. This can easily be collected in an inverted test-tube and shown to be pure oxygen.

CHAPTER VII.

THE MALE FERN (ASPIDIUM FILIX-MAS).

THIS is one of the commonest of all the British ferns, occurring most frequently in woods and shady situations throughout the whole of the British islands. The stem, or root-stock, from the summit of which the rosette of handsome green leaves or fronds grows, projects in a mature and well-grown plant some little distance above the ground.

1. Remove a single leaf, and note that it consists of a **Footstalk**, or **Petiole**, by whose base it is attached to the stem. A section of the petiole shows a nearly circular outline, and its lower part at least is completely covered over with large brown scales called **Ramenta.** Right and left of its insertion on the stem and extending up the petiole are two slight ridges which are lighter in colour than the rest of the dark brown surface of the petiole. These are the **Lateral Ridges**, or **Wings.** The petiole bears on its upper portion two rows of green lateral expansions, which are in the same plane as that containing the lateral ridges, and are called **Pinnæ.** These are not arranged opposite to each other, but they alternate one with another. Each pinna is also segmented quite, or almost, down to the middle line, and each segment, or **Pinnule**, is roughly oblong in shape and slightly toothed on the edge. Because the pinnæ are also pinnate, the whole leaf is said to be **Bipinnate.** Note that the segments of the pinnæ are finely veined by branches of an axial vein.

On the under sides of some leaves, which may be termed **Sporophylls**, are rounded bodies. These are **Sori**, arranged in a double row right and left of the central vein of each pinnule. With the point of a needle satisfy yourself that the visible portion is a thin kidney-shaped membrane attached to the leaf at one point not far from its centre. This is the **Indusium**, which covers and protects a powdery mass of **Sporangia** beneath it. On an older sporophyll the indusia show their edges recurved, thus exposing the sporangia.

2. Separate the leaves at the crown of the stem, and note developing leaves which are rolled up spirally with the apex within. The whole of such an immature leaf is covered with ramenta. At the apex of the stem the leaves are still less developed and completely cover the stem. It is three years from the first appearance of the rudimentary leaf to its complete unfolding. The leaf persists for one year, and then dies down to the swollen base of its petiole. This latter portion persists for many years, slowly decaying from above down to the stem.

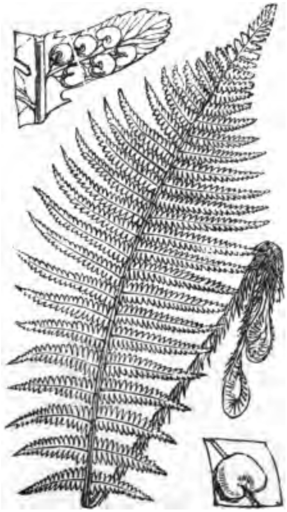

FIG. 56.—Leaf and sori of *Aspidium Filix-mas.*

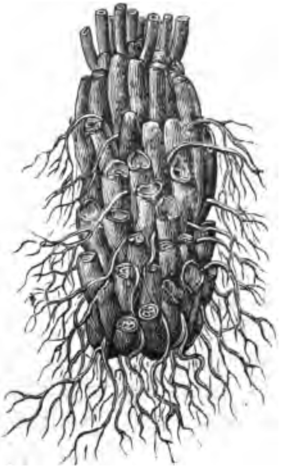

FIG. 57.—Stem of *Aspidium Filix-mas.*

3. Dig up a well-developed plant, and wash the stem well in water.

Note the roots, which proceed from all parts of the stem from the base to near the summit. They are thin, brown, wiry, with light-coloured apices, and are profusely branched. The stem proper is not visible, being covered below with the persistent bases of the leaves of previous years, and at the apex with those of the current year and with immature leaves. The latter with the apical portion of the stem form the **Apical Bud.** The stem grows erect, or at least but slightly oblique, and after removal of the bases of the petioles it appears to be larger towards the apex than at the base.

4. Cut the stem across horizontally about midway between its base and apex, and examine the cut edges after smoothing with a sharp razor.

The section is very irregular in shape owing to the attachment of the petioles. Note that the outer portion is covered with a thickish brown rind, from which arise numerous ramenta. The main portion of the substance of the stem—the **Ground Substance**—is light yellow in colour and somewhat friable. Darker spots appear on the section, the larger arranged in a ring. These are the sections of **Vascular Bundles.** The ground substance within this ring of bundles is known as the **Pith,** that outside as the **Cortical Parenchyma.** In the latter are seen sections of vascular bundles, branches of those of the main ring of bundles, on their way to the leaves. They are the **Leaf Trace Bundles.**

5. Make also a vertical section of the upper half of the stem, dividing it into approximately equal portions, and examine the section.

Note the cylindrical shape of the stem, the angle of about 60° which the petioles make with it, the ramenta which completely clothe stem and petioles, and form a close thicket on the flat apex in which are leaves in all stages of development down to mere slight projections of the apical tissue. Note that when they first appear the axes of the rudimentary leaves are perpendicular to the surface on which they originate, but that subsequently they suffer a displacement of 30°, their surface of origin, however, being displaced 90°. Mark the vascular bundles which are exposed. They do not form continuous series, and branches pass from them into the petioles (Fig. 59).

6. Commencing at the base of the stem, strip off the persistent bases of the petioles one by one. If they are bent back they snap off close to the stem.

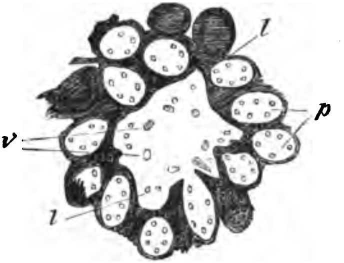

FIG. 58.—Transverse section of the stem of *Aspidium Filix-mas*. *p*, petioles; *v*, vascular bundles of the cylindrical network; *l*, bundles passing out to leaves and roots.

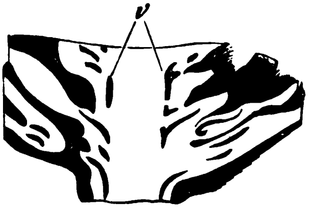

FIG. 59.—Portion of longitudinal section of the stem of *Aspidium Filix-mas*. *v*, vascular bundles.

A few bring away roots with them, but the majority of the roots have their origin in the stem. The leaf-scars (Fig. 60) now show that the leaves are arranged on the stem in a spiral. In very young plants the divergence is $\frac{1}{3}$, but as the plant gets older this arrangement of the leaves passes over into a $\frac{3}{8}$ arrangement, and this to $\frac{5}{13}$. This divergence is generally retained in the mature plant or passes over into $\frac{8}{21}$. At the apex proceed very carefully, and near its centre remove the ramenta one or two at a time with forceps. Then near

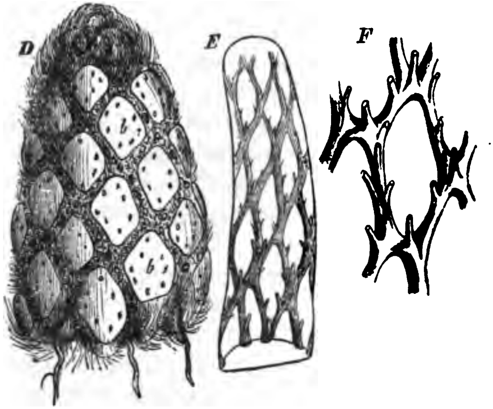

FIG. 60.—*Aspidium Filix-mas*. *D*, stem with leaf-bases removed; *E*, vascular bundles of the stem; *F*, a foliar gap.

the centre of the apex will be seen with a hand lens a small projection, the **Apical Papilla (Punctum Vegetationis,** or **Growing Point**), which terminates the apex of the stem.

7. Take one of the longitudinally divided halves of the stem, and, working at the cut surface with a blunt penknife or other instrument, prise away the pith until a semi-cylinder has been hollowed out exposing the vascular bundles. The ground tissue readily separates from the bundles although careful manipulation is needed at the apex.

The bundles are arranged in a cylinder of network with large open lozenge-shaped meshes. Each mesh is opposite the origin of a leaf, and is on that account called a **Foliar Gap**; the number of meshes therefore corresponds to the number of leaves. From the inside of the cylinder no branches are given off to the pith. At the apex the cylinder is nearly closed, being incomplete only below the apical papilla.

8. Working from the side, lay bare all the branches of the vascular bundle system which pass out into a leaf.

They are seven in number, all from the inner edges of the same mesh, one from its lowest angle, the others in pairs from higher up the mesh. The highest pair are the largest, and they enter and supply that side of the petiole which lies ventrally of the lateral ridges, the other five going into the dorsal side. Many other branches arise from the same mesh and pass out into roots.

9. Cut a root across and examine the cut surface with a hand lens.

One vascular bundle is seen occupying the centre of the root.

10. The roots branch very freely. Examine the apices of roots for lateral rootlets arising close behind them.

Note that a rupture of the tissue of the main root has taken place where a lateral root has arisen.

11. Take the persistent base of a petiole, make a clean transverse cut, and examine the exposed section.

Note first the yellowish brown rind, the ground-tissue as in the stem, and the exposed sections of the bundles—two larger on the ventral side and five smaller on the dorsal side.

12. Dissect away the ground-tissue, and lay bare the bundles throughout some portion of their length.

The five dorsal bundles anastomose freely with each other and less freely with the ventral bundles, *i.e.* they are connected together by frequent branches passing from one to another. The two ventral bundles are not connected by cross bundles. At the top of the swollen portion of the petiole the five dorsal bundles are reduced by fusions to three. In the lamina branches pass out into the pinnæ, and divide there as already described.

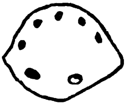

Fig. 61. — Transverse section of petiole of *Aspidium Filix-mas.*

13. Remove from the apex of the stem, and cut through longitudinally, leaves in all stages of development.

Note that the base of the petiole is first formed and is fairly well developed before there is any trace of lamina. Thus the growth of the leaf is apical, but limited. Leaves in a more advanced stage of development show the developing lamina rolled inwards on its axis in a spiral (in a **Circinate** manner). Note that whilst still tightly rolled up, and therefore in darkness, the lamina and axis show a green coloration.

14. Search for the persistent bases of petioles bearing buds on their dorsal sides, some of them with several leaves in course of development and two or three roots. Clean the base of the petiole and the lateral bud by removal of ramenta, and section through bud and petiole longitudinally.

Note the similarity, though on a smaller scale, of this bud to the apex of the main stem.

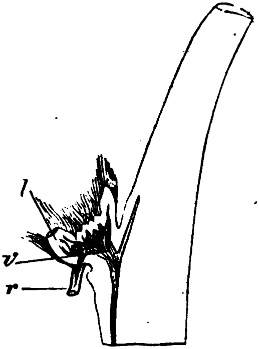

Fig. 62.—Longitudinal section of the petiole and bud of *Aspidium Filix-mas.* *v*, vascular bundles passing into the bud; *r*, young root; *l*, a leaf.

15. Take another one and dissect away the ground-tissue from petiole and bud, laying bare the bundle system.

It will be found that branches pass from one or more of the dorsal bundles of the petiole into the bud. Shortly after entering they unite to form a cup, and from the edge of this cup proceed branches which pass into the leaves and roots. If a transverse section be made through the bud stem close to the petiole the section of the bundle system will show a completely closed ring.

Microscopic Work.

For the investigation of the non-green tissues of the plant, alcohol material is very suitable, and in most cases desirable.

16. Trim off with a knife a portion of a petiole base containing rind, vascular bundle, and the ground-tissue between them. Cut very thin transverse sections of this. Select the thinnest sections, mount in glycerine, and examine first with a low and then with a high power.

Note first that the whole section appears made up of cellular elements. With the low power the whole elliptical outline of the vascular bundle is visible at once with its obviously dissimilar constituents. The ground-tissue shows rounded cells which, not coming in close contact with each other at all points, leave angular intercellular spaces. The rind is yellowish in colour outside, reddish-brown within, and consists of six or eight layers of cells, most of them with thickened walls. If a portion of a lateral ridge has been included in the section, it must be noted that the inner layers of the rind are discontinuous at that point, and the cells of the ground-tissue come nearly to the surface there. Examination with a higher power shows the following structures in order from without inwards :—

(*a*) An outer layer of cells, the **Epidermis,** the outer walls dark reddish-brown in colour, the lateral and inner walls being nearly colourless. All the walls are thin. Some cells of the epidermis are ragged where ramenta which are outgrowths of the epidermal cells have been torn away from them.

(*b*) A layer or two of cells with slightly thickened yellowish walls.

(*c*) Several layers of cells with reddish-brown strongly thickened walls. In very thin sections the colour is yellow. Contiguous walls show channels or pits which appear to connect the lumen of one cell with that of the next, but do not really do so.

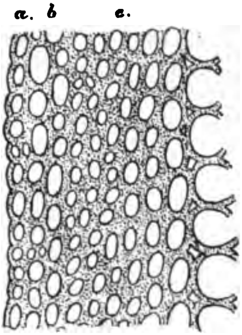

FIG. 63.—Transverse section through the rind of the petiole of *Aspidium Filix-mas*. *a*, epidermis; *b*, sub-epidermal tissue; *c*, sclerenchyma.

These cells form a tissue which is known as **Sclerenchyma.** The whole of the tissues *a*, *b*, and *c* constitute the rind already spoken of; *b* and *c* contain many starch-grains. The whole of the cells of the rind are in close contact with each other, and there are no intercellular spaces in it.

(*d*) The ground-tissue, **Parenchyma,** which consists of rounded cells with thin colourless walls and many angular intercellular spaces. The walls are profusely pitted wherever the cells are in contact, the pits, as in the sclerenchyma, extending down to the middle lamellæ of the cell-walls. In many parts of the section surface views of the cell-walls will be obtained, and there the pits are very evident. The outer walls of cells are very rough where they border on intercellular spaces. When first formed these cells are all in close contact at all points, but they early round themselves off and thus produce intercellular spaces. In many of the larger intercellular spaces are stalked **glands** with spherical heads attached to cells of the parenchyma. They contain resin. The cells of the parenchyma immediately surrounding the vascular bundle are smaller than those further away. The cells of the ground-tissue contain abundant protoplasmic contents and starch-granules.

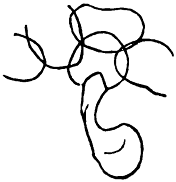

FIG. 64.—Internal gland of *Aspidium Filix-mas*.

(*e*) The **Endodermis** is a layer of cells which completely surrounds the bundle. The cells are of small width radially and are closely in contact with each other laterally. Their radial walls are so thin and easily collapsible that the cells are commonly compressed and difficult to make out.

(*f*) The vascular bundle has no intercellular spaces. It contains the following tissues:—The **Pericycle**, the **Phloëm**, the **Xylem.**

i. The **Pericycle** consists of several layers of parenchymatous cells, more or less angular, with thin colourless walls. At the ends of the major axis of the ellipse there are commonly but two layers of the pericycle, at the ends of the minor axis four to six. The cell-walls are colourless, and are deeply pitted. The outer layer of cells of this sheath correspond so closely to the cells of the endodermis that they are seen to have had a common origin. They all contain abundant protoplasm and starch.

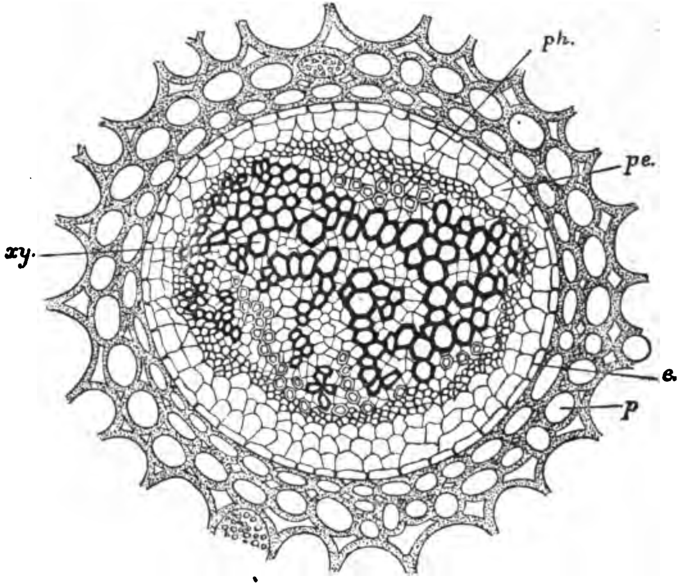

FIG. 65.—Transverse section through a vascular bundle of *Aspidium Filix-mas*. *p*, ground-tissue ; *e*, endodermis ; *pe*, pericycle ; *ph*, phloëm ; *xy*, xylem.

ii. Next comes the **Phloëm**, the elements of which are of three forms. On the outside, next to the pericycle, are **Bast**

Fibres, sometimes called **Protophloëm**, as being first formed. Each shows a much thickened colourless wall with lumen almost obliterated. They are the only elements of the phloëm which are found at the extremities of the major axis of the ellipse. Then the openings of the **Sieve-tubes** are to be noticed. They have colourless walls, and are slightly larger than the cells among which they lie. Their only contents are a thin lining of a highly refractive glistening substance. Around and amongst the sieve-tubes are parenchymatous cells.

iii. The **Xylem** contains the largest elements of the bundle —large polygonal outlines with much thickened walls of light yellow colour. These are **Tracheides**, amongst which lie many parenchymatous cells containing protoplasm and starch. These are sometimes called **Packing Cells.** A single layer of parenchymatous cells also completely surrounds the xylem. The tracheides have no contents.

Such a vascular bundle as that now described is a **Concentric** bundle. The xylem is completely surrounded by the phloëm.

17. Mount a similar section in Schultze's fluid.

Note that the walls of all the parenchymatous cells of the section, as well as those of the sieve-tubes, are coloured violet, showing the cellulose reaction. Starch appears in the ground-tissue, pericycle, and the parenchyma of the xylem; not, however, in all the cells between and amongst the sieve-tubes. Some of them, the **Companion Cells**, show much protoplasm but no starch.

18. Mount another section in acid aniline chloride.

The walls of the tracheides take on a beautiful golden yellow colour. This is the reaction for **Lignin.** The walls of the sclerenchyma are also lignified, but the reaction is not so evident here on account of their deep colour.

19. Place a section in strong sulphuric acid and cover with a cover-glass.

The effect of the strong acid is to completely disorganize the whole section. Walls of cells and vessels swell up and their outlines disappear. The only portions unaffected are the radial **Corky Walls** of the endodermis, which now show up as clearly defined, thin, wavy lines. If the section is not too thin, pressure on the cover-glass will bring these walls to the surface and show them in their tangential aspect.

Very beautiful and instructive preparations of the bundles may be made by injecting them with colouring matter in the following simple manner:—Slip the end of a piece of india-rubber tubing about five or six inches long over the end of a persistent base of a petiole—alcohol material is the best—fill up the tubing with an alcoholic solution of fuchsine, and screw a small clamp on the other end. Place something on the middle of the tubing so as to exercise a steady gentle pressure and allow to remain for an hour or so. On removing the tube it will be found that the colouring matter has passed deeply into the bundles, but to a very slight depth in the ground-tissue. Cut sections from this, and mount in glycerine. The various elements of the bundle are rendered beautifully distinct by this method. The contents of all the protoplasm containing cells within the bundle, as well as the walls of the tracheides, will be stained, but no colour passes through the endodermis.

20. Lay some sections in Eau de Javelle for an hour or so. This reagent dissolves out the whole of the cell contents. Wash well in dilute acetic acid, and mount in glycerine; or stain with fuchsin and lay in glycerine for some hours, when the colour will be removed from all but the lignified and corky cell-walls.

Such cleared divisions show the outlines of the cells well. It can now be seen that the cells of the endodermis correspond so closely with the outer layer of cells of the phloëm-sheath, that there is no doubt they have a common origin.

21. Prepare longitudinal radial sections through rind, ground-tissue, and bundle, and treat as with transverse sections. The details of cleared sections will be best made out.

The epidermal cells are now seen to be several times longer than broad, and with blunt ends. The cells of the sclerenchyma are long, with pointed ends, and their thick walls are deeply pitted down to the middle lamella. There is a quick transition from these pointed cells to the short, blunt, thin and pitted-walled parenchymatous cells of the ground-tissue. In a thin and well-cleared section there will be no difficulty in making out the long narrow cells of the endodermis, in shape and size nearly resembling those of the epidermis. Next come the deeply pitted parenchymatous cells of the pericycle, possibly some sharp-pointed bast fibres, and then long sieve-tubes with delicate wavy walls. Much searching with a high power will probably be necessary before delicate pits in circumscribed **Sieve-areas** are to be detected on their lateral walls. Then comes the parenchyma surrounding the tracheides, and finally in the middle of the bundle the tracheides themselves. Note that they are many times longer than broad, and most have pointed ends. They are marked with peculiar transverse stripes like the rungs of a ladder, and are on that account called **Scalariform** tracheides. These stripes are deep pits in their wall. Outgrowths of the walls around the pit meet nearly in the middle line, so that the pit is wider at the bottom than at the top, and it is on that account called a **Bordered Pit.** Where one tracheide is in contact with another the pits in the two are opposite to each other; where a tracheide borders on a parenchymatous cell the common wall is pitted only on the side of the tracheide.

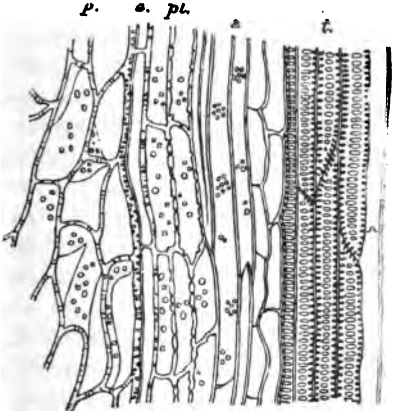

FIG. 66. — Longitudinal section through a small vascular bundle in the stem of *Aspidium Filix-mas*. *p*, ground tissue; *e*, endodermis; *pl*, pericycle; *s*, sieve-tubes; *t*, tracheides of the xylem.

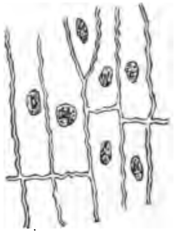

FIG. 67. — Endodermal cells of *Aspidium Filix-mas* with wavy radial walls and large nuclei.

22. It is not easy to cut accurate tangential sections so as to get surface views of the endodermal cells, but frequently a longitudinal section through a bundle passes out of the middle line so far as to expose the surface of the endodermis. Search for such a section, and note the wavy radial walls of the endodermal cells and their large and distinct nuclei.

23. Isolate portions of vascular bundles and place them in a test-tube with a few crystals of potassium chlorate and strong nitric acid. Heat until bubbles of gas are formed, and then fill up with cold water to stop the action. Or, better still, allow the whole to remain at the ordinary temperature for a week or two. Then place a small portion on a slide in a drop of water, tease it out with needles, and examine it either in water or in glycerine. The effect of this maceration treatment is to dissolve out the middle layers of the cell-walls and isolate the cells.

Note the completely isolated tracheides, and their pointed ends which are pitted in the same manner as their side walls. Not unfrequently individuals are seen with forked ends. Look out for **Spiral tracheides,** *i.e.* with their walls thickened by spiral bands. These are the first formed elements of the xylem, and lie at the poles of the elliptical bundle. The long bast fibres with pointed ends are very evident. The parenchymatous cells form filaments, each of six or eight cells, placed end to end, the terminal cells being pointed at their free extremities; each group is formed by the transverse

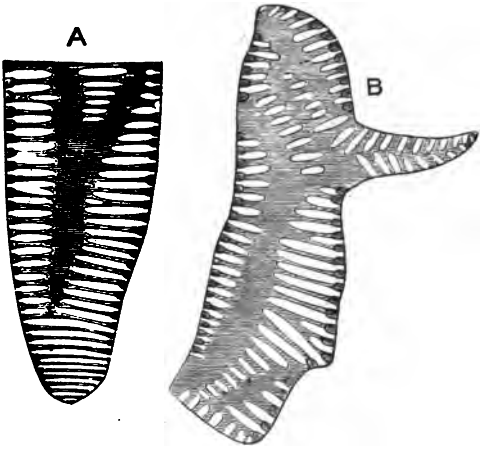

FIG. 68.—*Aspidium Filix-mas.* A, end of a scalariform tracheide; B, whole tracheide with branching process.

division of a long and pointed mother-cell. The sieve-tubes are recognizable only by their scanty contents being contracted into a slender cord running through the middle of the tube.

Sections through the stem will show the same tissues in the same order as in the petiole. The bundles, however, are larger, and on account of their frequent branching are more complicated.

24. Cut transverse and longitudinal sections through a very young bundle near the apex of the stem. Clear with potassium hypochlorite, stain with fuchsine, and lay in glycerine for a time. Then mount in glycerine.

If the bundle is not too young the spiral tracheides which form the **Protoxylem** will be well stained and they alone. The centre of the bundle is made up of long, thin-walled, and pointed cells, which later become developed into the various elements of the bundle.

25. Clear the apex of a stem and lay bare the apical papilla. Cut out with a keen knife a conveniently shaped wedge of the apical tissue containing the apical papilla, and from it cut longitudinal sections. Alcohol material should be used. Select the section which appears to be most median, clear in potassium hypochlorite, and mount in water or glycerine. The student is warned that it is not an easy thing to get a good median longitudinal section, and he must be prepared for many failures. Examine with a high power.

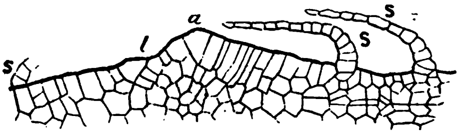

FIG. 69.—Stem apex of *Aspidium Filix-mas*. *a*, apical cell; *l*, origin of leaf; *s*, scales. (Hofneister.)

The actual apex is occupied by a tetrahedral cell whose base is convex and exposed. From this, by cell-walls parallel to the sides of the pyramid, successive cells are cut off, so that all those below the apical cell are its daughter-cells. Further divisions in these differentiate the apical tissue into three groups—

i. An outer layer, from which the epidermis of the stem is developed. These cells divide mainly by radial walls. Some however, divide by tangential walls, and give rise to the ramenta which thickly clothe the epidermis.

ii. A central bulky mass of tissue, forming cell-walls in all planes, the cells early partially separating from each other and forming intercellular spaces.

iii. Running through this tissue strands of long narrow cells, which later develop into vascular bundles.

Internal glands are early formed in the intercellular spaces, and appear a very short distance behind the apex.

26. Lay bare an apical papilla, and remove its apex by a transverse cut with a sharp razor. Mount in water and examine with a high power. If the section is too opaque lay it in potassium hypochlorite for a very short time, and mount in glycerine.

Note that the section of the apical cell is triangular and the cells around it show by their relative positions that they have been cut off from it by walls parallel to its three sides. From the longitudinal and transverse sections it is clear that the apical cell is a three-sided pyramid, and that cells are successively cut off from it by walls parallel to its three sides.

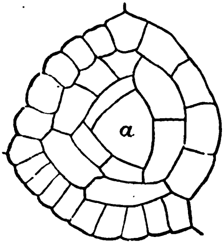

FIG. 70.—Transverse section through the apical cell, and its segments, of *Aspidium Filix-femina*. *a*, apical cell. (After Naegali and Leitgeb.)

Examination of the rudiments of leaves shows that they have two-sided apical cells, from which segments are alternately cut off from opposite sides.

27. Strip off a piece of epidermis from the upper side of a pinna of a leaf and mount in water.

Note that it consists of large cells with sinuous outlines and abundant chlorophyll bodies. The cells are in close contact with each other. There are no intercellular spaces.

28. Do the same from the under side of a pinna.

There are cells of two kinds. Firstly, sinuous cells like those of the upper epidermis, and without intercellular spaces; secondly, crescent-shaped cells in pairs, showing a cleft between them. These latter constitute the **Stomata.** The crescent-shaped cells are the **Guard-cells**, which are in contact with each other at their extremities, but are separated more or less widely in the centre. This **Stomatic Cleft** places the intercellular spaces of the leaf in connection with the external air. The guard-cells also contain chlorophyll.

29. Cut off the tip of a pinnule and mount in water. Apply slight pressure, and air will escape from the cut edge. Examine with a low power.

The intercellular spaces are numerous and full of air, and the leaf is nearly opaque. Where air has been squeezed out it can be observed, by deeper focussing, that cells lying below the surface also contain chlorophyll bodies. Special examination will show that they have grains of starch in them as a rule.

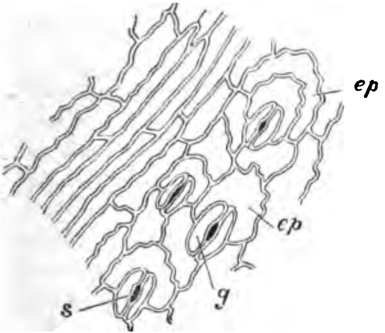

FIG. 71.—Stomata in leaf of *Aspidium Filix-mas*. *s*, stomatic cleft; *g*, guard-cell; *ep*, epidermal cell.

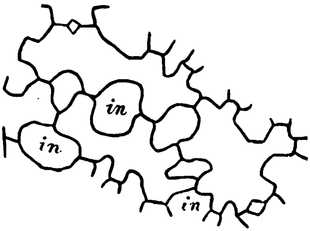

FIG. 72.—*in*, intercellular spaces in the mesophyll of the leaf of *Aspidium Filix-mas*. View obtained by focussing below the surface.

30. Lay a similar piece in potassium hypochlorite for a time until transparent; then mount in glycerine, and examine first with a low and next with a higher power.

Note that the long axes of the stomata are parallel to the axis of the pinnule. With the lower epidermis uppermost, focus beneath it, and note that cells next succeeding are irregular in shape, with peg-like projections which attach them to the epidermis and to each other. Amongst them are consequently many intercellular spaces. The cells immediately beneath the upper epidermis are not so irregular in shape, but are much more closely packed. The chlorophyll-containing tissue lying between the epidermal layers is known as the **Mesophyll.** Running through the mesophyll are the vascular bundles. Above and beneath them the cells of the epidermis are elongated in the direction of their length and have no stomata. Get optical sections, and note that the simpler bundles have a few spiral and scalariform tracheides surrounded by delicate elongated cells. Note especially the terminations of the bundles a little short of the teeth of the pinnule. At the extremity each bundle is swollen, the number of tracheides there is much increased, and some one or two appear to project beyond the other elements of the bundle.

31. Cut transverse sections across the pinnule. For this purpose leaves which have been kept in alcohol until somewhat crisp are best. Pack several together all the same way, place within a piece of split pith and cut. Clear in potassium hypochlorite, mount some in water. Treat others with Schultze's fluid, with acid chloride of aniline, and with strong sulphuric acid. Examine with a high power.

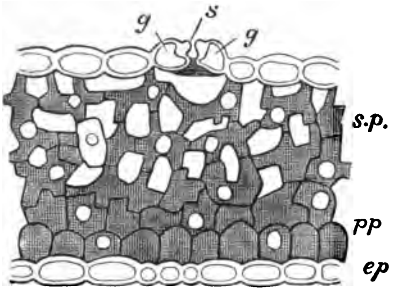

FIG. 73.—Transverse section of a leaf of *Aspidium Filix-mas*. *ep*, epidermis of the upper surface; *s*, stoma; *g*, guard-cells; *pp*, palisade parenchyma; *s.p*, spongy parenchyma.

The leaf is thickest through the mid-rib. This projects strongly on the under side, a corresponding depression being found on the upper side. At these places it is strengthened by several layers of sclerenchymatous cells. The bundle runs through the centre, and is of the same character as those already described and of which it is a branch. The rest of the leaf is of about equal thickness throughout, the mesophyll consisting of four or five layers of large cells. The irregularly shaped cells of the lower spongy portion and their connecting processes are now clear. Since the processes project in all directions, some will be above or below the plane of the section, and these cells will appear as if there were holes in them (Fig. 73). The ends of no processes are free; all are united to processes of other cells.

Look along the lower epidermis for transverse sections of stomata. These will now be seen to rise above the level of the epidermis. The guard-cells have irregularly thickened walls. Two ridges run along the concave faces of each, a groove lying between them, and the corresponding ridges of the two cells are opposed to each other. The two outer ridges border the **Entrance**, the two inner the **Exit**, the groove between them is the **Front Cavity,** and the intercellular space immediately beneath the stoma is the **Respiratory Cavity.** Some of the sections will show longitudinal sections of stomata, and from these it can be seen that the guard-cells are concave towards the mesophyll.

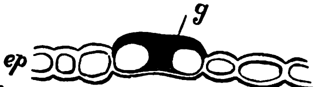

FIG. 74.—Longitudinal section through the cleft of a stoma from a leaf of *Aspidium Filix-mas*. *ep*, epidermis; *g*, guard-cell.

Schultze's fluid stains the cell-walls of the section blue (cellulose reaction), with the exception of the sclerenchyma, the tracheides of the vascular bundle, the endodermis, and the outer layer of the outer wall of the epidermis. This latter layer is the cuticle. It covers the whole epidermis, including the guard-cells, and extends over the entrance of the stoma. Sulphuric acid dissolves everything but the cuticle and the endodermal cell-walls. Acid aniline chloride shows that the tracheides and sclerenchyma are lignified.

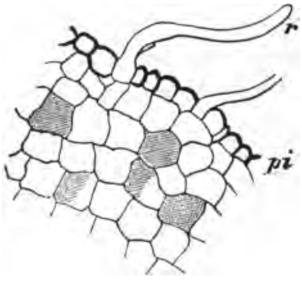

FIG. 75.—Transverse section through the outer layers of a root of *Aspidium Filix-mas*. *pi*, piliferous layer; *r*, root-hair.

32. Cut transverse sections of a root. Mount some in water, clear others with potassium hypochlorite, and mount in glycerine. Examine with a low and with a high power.

Note the irregular brown **Piliferous Layer,** with some of its cells growing out into long unicellular hairs. Next to this is the thin and pitted-walled cells of the parenchyma of the root. Where a surface view of a cell-wall is obtained it is clearly striated obliquely in two directions. The parenchyma has no starch-grains. It passes over rather suddenly into a sheath, many layers thick, of sclerenchyma surrounding the axial bundles which occupy the axis of the root. In cleared specimens the endodermis is evident. It is similar to that of the stem. At opposite ends of the ring of endodermis near the protoxylem of the bundle are single cells, the so-called **Rhizogenic Cells,** which are larger than all the others of the ring. In cells of the endodermis like these lateral roots have their origin. Next to the endodermis comes the pericycle, consisting of a layer, in some parts two, of parenchymatous cells; then the phloëm, alternating with the xylem and containing many sieve tubes with polygonal outlines; and finally the band of xylem. The largest tracheides are in the centre, and they are also the youngest. At the poles of the xylem are the two groups of protoxylem elements consisting of a few spiral tracheides. These are the first formed, and

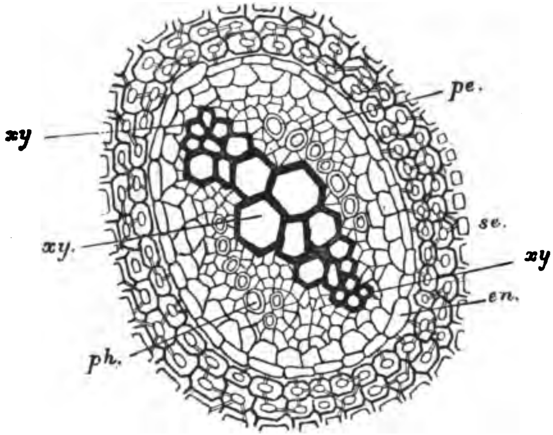

FIG. 76.—Transverse section through the central cylinder of a root of *Aspidium Filix-mas*. *se*, sclerenchyma; *en*, endodermis; *pe*, pericycle; *ph*, phloëm; *xy*, xylem.

they are subsequently united by larger scalariform tracheides. Because there are two masses of protoxylem, the bundle is said to be **Diarch.**

Schultze's fluid shows that the cell-walls of the cortical parenchyma, the phloëm, and pericycle are of cellulose, although the coloration is not immediate. Strong sulphuric acid shows that the endodermal cell-walls are corky, and that there are also corky cell-walls in the cortical parenchyma just beneath the piliferous layer. These cells form the **Exodermis.** Acid aniline chloride demonstrates that the walls of the sclerenchyma, as well as those of the tracheides, are lignified.

33. Cut longitudinal sections of a root, treat as above, and examine with a high power. Note the following.

The cells of the piliferous layer are several times longer than broad. The cortical parenchyma cells show their delicately pitted walls with small intercellular spaces. The sclerenchyma cells are long and pointed and deeply pitted with oblique pits. The elements of the bundle are similar to those of the stem bundles.

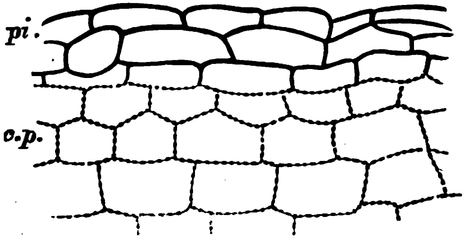

FIG. 77.—Longitudinal section through the outer layers of the root of *Aspidium Filix-mas*. *pi*, piliferous layer; *c.p*, cortical parenchyma.

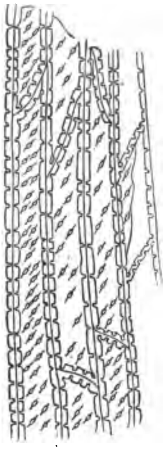

FIG. 78.—Longitudinal section through the cells of the sclerenchymatous sheath in the root of *Aspidium Filix-mas*.

34. Cut longitudinal sections of the root-tip, using material hardened in alcohol. To do this place it between pieces of pith, soft cork, or some succulent substance such as carrot, and cut through the whole, or fix it on the smooth surface of a cork with gum or coaguline and cut in alcohol, or, best of

all, with a solution of celloidin in a mixture of equal parts of absolute alcohol and ether, and cut in alcohol. Clear the sections with potassium hypochlorite or potash solution, mount in water or glycerine. With a low power select that section which appears to be most nearly median, and then examine it with a high power.

In the median section observe that the extremity of the root is occupied by a mass of tissue which caps the root proper, and which is on that account called the **Root-cap.** Its outer cells are constantly wearing off and being replaced by others which are the daughter-cells of the **Apical cell** of the root. This lies embedded in the tissue at the base of the root-cap. Like the apical cell of the stem, it is a three-sided pyramid, but unlike that it forms division walls in four planes — three parallel to the sides of the pyramid, and one parallel to its base, which is turned towards the root-cap. Segments parallel to the sides give rise by their divisions to the tissues of the root, the segments parallel to the base to the root-cap. At first the **Meristematic** cells are all alike, but a short distance behind the apex the cells at the centre lengthen out. From these later on are formed the elements of the axial bundle.

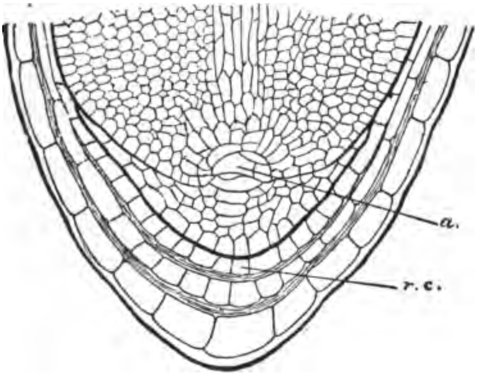

FIG. 79.—Root-apex of *Aspidium Filix-mas*. *a*, apical cell; *r.c.*, root-cap. (Hofmeister.)

35. Cut transverse and longitudinal sections of a root a short distance behind the root-tip. Clear with potassium hypochlorite, stain with fuchsine, and lay in glycerine for a time. Then mount in glycerine.

Only the tracheides of the two groups of protoxylem are stained. The large scalariform tracheides may be present but are not yet fully formed, nor are their walls lignified.

36. Isolate the elements of the root by maceration with potassium chlorate and nitric acid.

There is nothing special to note except that it can now be seen that the root-hairs are produced, not from the whole external surface of the epidermal cells, but from only a limited portion of each. The other elements correspond to those of the stem.

Reproduction.

As will be seen later on, the whole of the cells constituting the fern plant are the direct descendants of a single cell, the **Oosphere.** All the members of its numerous progeny in early life, whilst constituting the meristem at its various growing points, have the power of division. In normal growth the vast majority by a limited number of divisions exhaust their power, and are then converted by growth alone into the different elements of the permanent tissues of the stem, root, and leaves. A few, the apical cells of the leaves, retain it for a longer, although still a limited period. A smaller number still, like the apical cells of most buds, hold the power in reserve, and after a dormant period exercise it or not as the case may be. A very few, the apical cells of the stem, growing buds and roots, retain it and exercise it until, so far as is known, inhibited by conditions lying outside themselves. The apical cell of the stem or growing bud retains its individuality and power of producing cells, and thereby giving rise to new stems, leaves, and roots for a period, of which the limit, if there be one, is unknown, but is certainly very great. After the formation of every daughter-cell, the mother-cell grows to its original size before another cell is cut off from it. The activity of all these dividing cells is, however, dependent, amongst other conditions, on their retaining their natural connection with at any rate a large portion of the plant, for none of them are capable of assimilation, and must therefore draw their plastic substances for growth from the plant's stores of food, which we have seen are the stem and the swollen petiole bases.

But the plant produces other cells, each containing its own store of food, and each severing its connection with the plant and carrying on an independent existence. These cells are the **Spores** which, under favourable conditions, grow and divide, thus producing other cells. They do not, however, develop directly into fern plants.

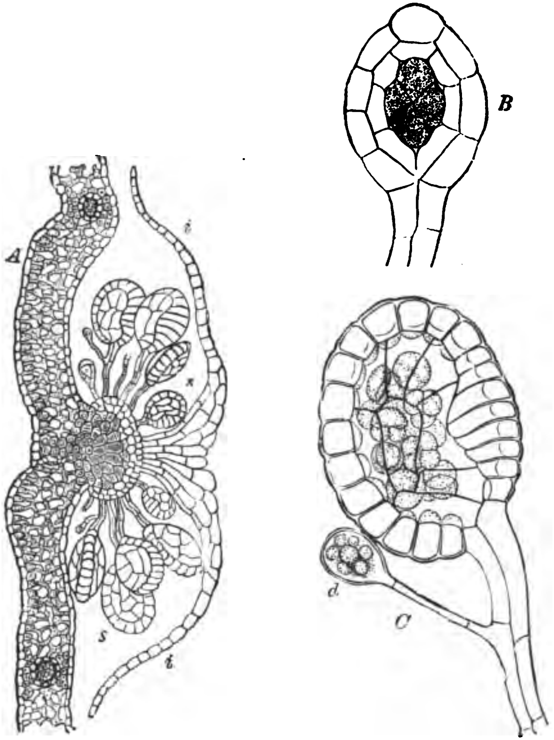

FIG. 80.—*Aspidium Filix-mas*. *A*, section through leaf and sorus; *s*, sporangia; *i*, indusium; *B*, young sporangium; *C*, mature sporangium containing spores; *d*, glandular hair. (Sachs.)

37. Select a young, still rolled-up leaf, slit it longitudinally, and examine the young pinnæ with a hand lens for developing sori on their lower surfaces. Cut thin transverse sections by imbedding a suitable piece in solution of celloidin, placing on

a cork, and cutting, when sufficiently set, in alcohol. It will generally be found necessary in this, as in other cases where meristematic tissues are cut, to clear in potassium hypochlorite. Mount in glycerine, and examine with a high power.

Look for transverse sections of sori. If at a suitable stage each will be found to consist of a small hemispherical mass of tissue, the **Placenta,** on the summit of which is the umbrella-shaped **Indusium** (Fig. 80, *i*). From superficial cells of the placenta are developed the **Sporangia** in the following manner: The cell divides by a transverse wall, and the outer cell so cut off is the mother-cell of the sporangium. This also divides by a transverse wall, and from the lower of the two cells so formed arises the stalk, from the upper the head or capsule of the sporangium. The basal or stalk cell by growth and division forms a stalk of two or three rows of cells. Frequently one of its cells produces a glandular hair similar to those already described. The upper cell divides first into four outer cells, enclosing a single cell, the **Archesporium.** The outer cells form the envelope of the capsule, dividing by walls perpendicular to the surface until a lenticular body is produced, the rim of which is formed by a ring of cells with thicker walls than the others, and projecting beyond them. This ring is the **Annulus.** The archesporium first cuts off an outer layer of cells, the **Tapetum**; and at this stage the capsule consists of an outer envelope, the wall of the capsule, an inner envelope, the tapetum enclosing a single cell. This latter then divides up into usually sixteen **Spore-mother-cells,** whilst the cells of the tapetal layer are dissolved, and the spore-mother-cells float free in the liquid which fills the capsule. Each spore-mother-cell forms four

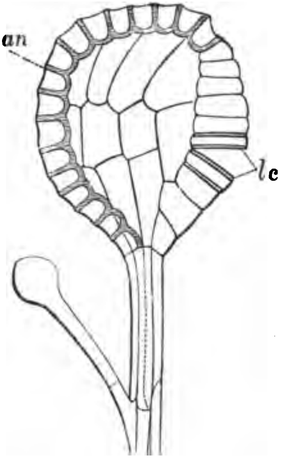

FIG. 81.—Sporangium of *Aspidium Filix-mas*. *an*, annulus; *lc*, lip-cells. (Kündig.)

spores, the nucleus dividing first, each of which has a double coat, the outer of which, the exospore, is corky and much corrugated in the ripe spore. The sporangium opens when ripe and sheds its spores by the action of the annulus. On drying up this flattens somewhat and is ruptured transversely. The rupture commences between two **Lip-cells** with lignified cell-walls.

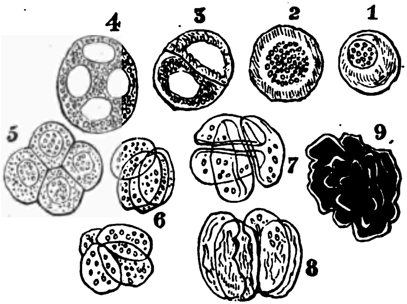

FIG. 82.—Development of the spores of *Aspidium Filix-mas*. 1–9, order of development. (After Sachs.)

In sections of a mature sorus it will be seen that it lies over a vascular bundle, the placenta being full of short tracheides.

When studying sections of young pinnæ the student should not miss the opportunity of studying the development of the stomata, each from a single cell cut off from an epidermal cell, as well as the apical cell of the pinnæ and leaf.

38. Take a flower-pot saucer, make a hole in it, half fill it with a layer of peat, press this to a fairly smooth surface, and shake over it the leaf of a plant which is discharging spores. Keep it moist and out of direct sunlight. Watch it from day to day with a hand lens, and remove and examine germinating spores in all stages of development. The earliest stages are best seen in spores germinating in a hanging drop of water in a moist chamber. The stages of development are as follow :—

The exospore bursts and the endospore protrudes as a tube. Transverse divisions appear, and a filament of three or four cells is formed. Then longitudinal divisions occur, and the extremity broadens out into a flat expansion of cells, the **Prothallium**, containing each a large nucleus and many chlorophyll bodies. At first there is a distinct apical cell, but this gives place to a depressed apical region occupied by many closely packed columnar meristematic cells. Many cells on the under side grow out into **Rhizoids.** The mature prothallium is roughly heart-shaped—several layers of cells thick behind the apex—has no intercellular spaces and no stomata, and grows obliquely upwards in a diffused light; in bright sunlight it clings closely to the substratum.

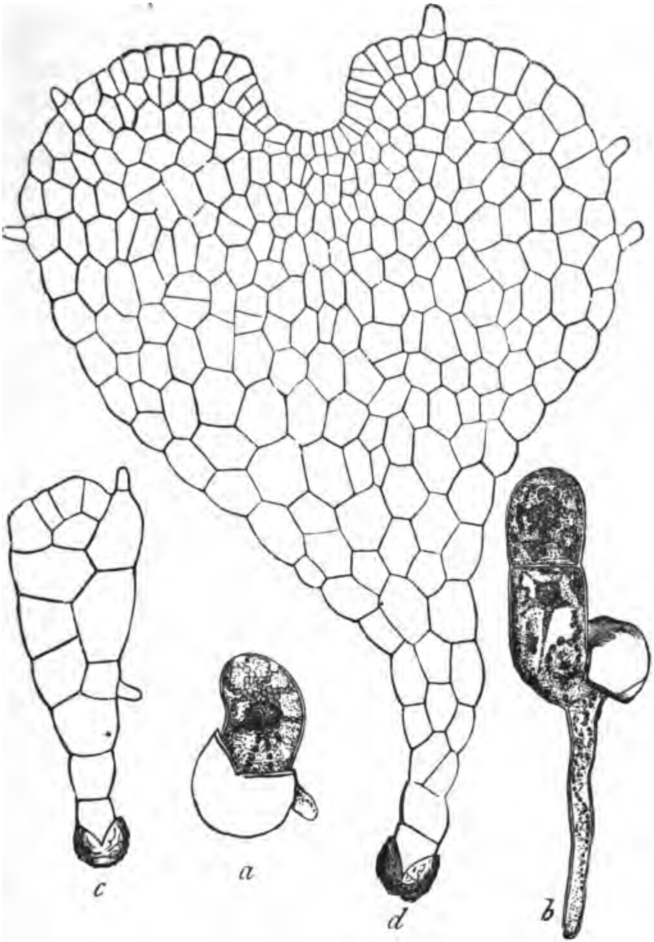

FIG. 83.—Germination of a fern spore. *a, b. Dicksonia antarctica; c, d, Aspidium Filix-mas.* (Luerssen.)

39. Remove a mature prothallium, wash it, mount it in water, and examine its under surface, first with a low and then with a high power.

Note the points already mentioned. Some cells at the periphery are grown out into glandular hairs. Look for the **Sexual Organs**—the **Archegonia** on the thickened cushion of the prothallium in the middle line just behind the growing region, and the **Antheridia** behind amongst the rhizoids. Both project from the surface, and, in order to understand their structure, lateral (or sectional) views are necessary as well as surface views. They may be got thus: Fold a mature prothallium back upon itself along the middle line from apex to base. Mount it in water, and the sexual organs will be seen in profile along the ridge of folding. Another and better method is to harden mature prothallia in alcohol, pack them together in the manner directed for the leaves, and cut sections.

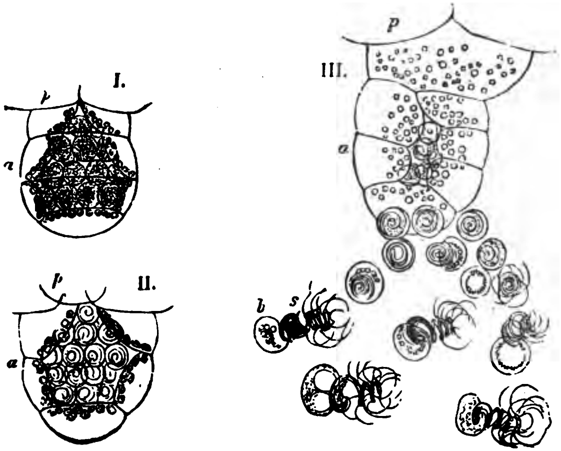

FIG. 84.—Antheridia of *Adiantum capillus-Veneris* in different stages of development. *p*, prothallium; *a*, antheridium; *s*, antherozoids; *b*, vesicle with starch-grains.

The development and structure of these organs can be fairly well followed by the student.

The antheridium first appears as a hemispherical projection of a superficial cell of the prothallium on its under surface.

A transverse wall cuts it off from its parent-cell, and it is then divided into an angular central cell surrounded by a layer of chlorophyll-containing cells in two or three stages. One of these covers the extremity of the antheridium like a cap. The central cell divides up into a number of small cells which are the mother-cells of the **Antherozoids.** From their nuclei are formed the spirally-coiled antherozoids, with cilia at their anterior extremities. In living prothallia, mounted in water, the bursting of the antheridium and the escape of the antherozoids can be followed. The peripheral cells absorb water, swell up, and burst the cap-cell in a stellate manner. From the rupture are pressed out the antherozoids, each in its little **parent-cell.** They soon escape from these, and move about in the water by the action of their cilia, each spiral antherozoid rotating on its axis during locomotion. Motion continues for about half an hour. Small vesicles containing granules, the remnants of the parent-cells, remain attached to the posterior extremity for a longer or shorter period, to be finally shaken off.

The archegonium is also developed from a superficial cell. This projects and is divided into a row of three cells by transverse walls. The **Basal Cell,** the one in contact with the prothallium, forms the cells constituting the **Venter** of the archegonium, sunk in the tissue of the prothallium and enclosing the **Oosphere.** The terminal cell divides up into four rows of cells to form the neck of the archegonium. The middle cell pushes its way downwards between the neck cells, and upwards between the cells of the venter, and divides into three —the large oosphere lying in the venter, the **Ventral Canal-**

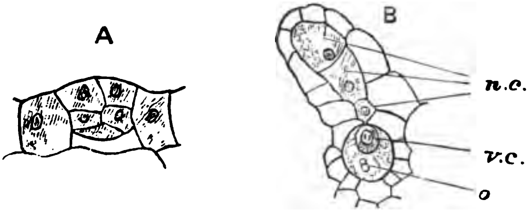

FIG. 85.—Archegonia of *Aspidium Filix-mas.* A, young; B, nearly mature; *o*, oosphere; *v.c*, ventral canal-cell; *n.c*, neck canal-cells. (After Hofmeister.)

cell also in the venter, and a long narrow **Neck Canal-cell** filling up the neck of the archegonium. When this is ready for fertilization the walls of the two canal-cells are converted into mucilage, the neck of the archegonium opens at the apex, and the mucilage with the protoplasm of the canal-cells is discharged. If antherozoids are being set free close at hand, and the under side of the prothallium is covered with a layer of water, they make their way to the mucilage, being attracted by salts of malic acid which it contains, and crowd up the neck of the archegonium. One enters the oosphere at a spot which is less granular than the rest, called the **Receptive Spot.** The neck of the archegonium is convex towards the anterior extremity of the prothallium, and is therefore bent back towards the antheridia. This facilitates the entrance of antherozoids. In spite of this, extremely few oospheres are impregnated, absence of water being of course a fatal defect.

After impregnation, the **Oospore** commences to divide and form an **Embryo.** The first wall formed contains the axis of the archegonium, but is perpendicular to the axis of the prothallium. This is the **Basal Wall,** which divides the oospore into an anterior half turned towards the growing region of the prothallium, and a posterior half. Then two other walls are formed perpendicular to the basal wall and to each other. These are the **Median Wall,** which contains the axis of both archegonium and prothallium, and the **Transversal Wall,** which is parallel to the surface of the prothallium, and divides the oospore into an upper and a lower half. These three walls divide up the oospore into octants,—two upper and two lower anterior, and two upper and two lower posterior. The student should round off the ends of a cork, cut it into octants, and mark them as indicated.

From one of the upper anterior octants is formed the growing point of the stem with its apical cell, the other does not develop. The two lower anterior octants develop into the first leaf, the **Cotyledon.** One of the lower posterior octants forms the first root, the other does not develop; the two upper posterior octants develop into the **Foot,** which consists of a mass of tissue remaining in the prothallium, and absorbing from it nourishment to feed the young plant until it can get its own living. It should be noted that the first leaf and root arise independently of the stem, all subsequent ones being developed from it.

Should an archegonium be fertilized, the prothallium makes but little subsequent growth and soon dies away, but if unfertilized, then a prothallium can, under favourable circumstances, lead an independent life for a considerable period. The prothallia of some ferns increase by vegetative budding, these buds being detached and carrying on a separate existence. It has been seen that the prothallium of *Aspidium Filix-mas* has taken the first step towards the diœcious condition, inasmuch as antheridia are first produced, and then archegonia. But prothallia of ferns are known which are completely diœcious, some bearing only male organs, and others female, although both kinds are produced from spores which are apparently alike in all respects. It must be mentioned here, also, for the proper understanding of the flowering plant, that there are fern-like plants higher in the scale of life than our fern whose diœcious prothallia are formed from unlike spores—male prothallia from small spores called **Microspores**, and female prothallia from large **Macrospores.**

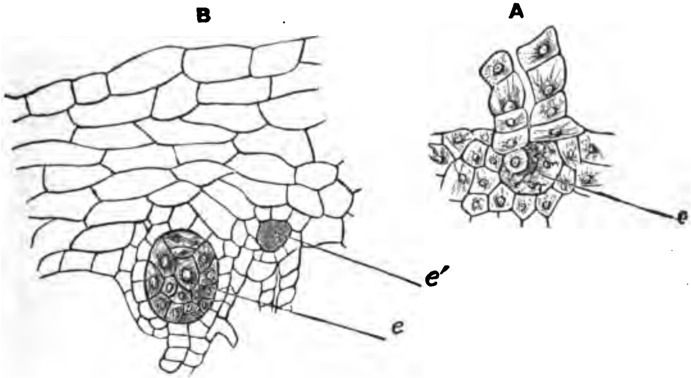

FIG. 86.—Archegonia of *Aspidium Filix-mas*. A, with antherozoids in the oosphere; B, formation of the embryo; *e*, after fertilization; *e′*, is an oosphere which has escaped fertilization. (After Hofmeister.)

For a time the young embryo remains imbedded in the tissue of the prothallium. Soon, however, the cotyledon and root burst through, the root growing downward and the leaf and stem turning upward. The first leaf is very imperfect, and it is not until several have been produced that they are fully formed. Each year's increment of the stem is larger in diameter than the previous year's, so that for a time it is conical with the apex of the cone downward; but a time comes when this is not so, and it grows fairly cylindrical. There is no means by which any portion of the stem, once fully formed, can go on increasing in thickness.

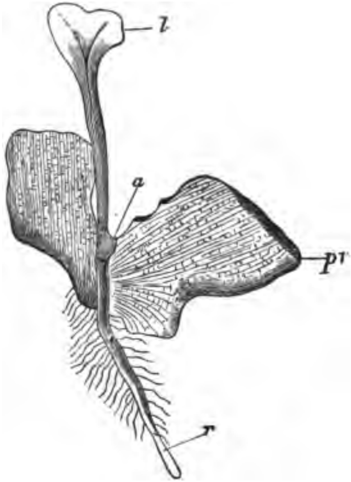

FIG. 87.—Young plant of *Aspidium Filix-mas* still attached to the prothallium, *pr.* *l*, first leaf; *a*, stem apex; *r*, root.

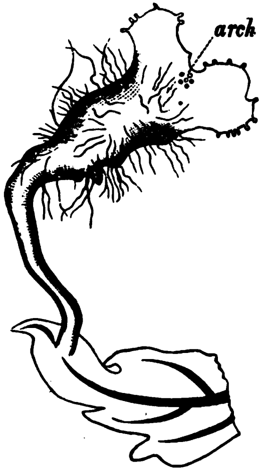

FIG. 88.—Prothalloid growth of *Polystichum angulare*, var. *pulcherrima*, on the surface of a frond; *arch*, archegonia. (Bower.)

The stem of the young plant has at first but a single axial bundle, to which the single bundles from the leaves are united. At the fifth or sixth leaf the single axial bundle opens out into the network characteristic of the mature stem. Still, however, but one bundle passes into each leaf, that namely from the lower angle of its own foliar gap. In its second year, as the divergence still further increases, the leaves are stronger and more vascular bundles pass into them.

The alternation of generations in this plant is clear and distinct. That which is commonly called the fern plant is the **Sporophyte**, bearing only spores and no sexual organs; the prothallium is the **Gametophyte**, or **Oophyte**, the sexual-organ-bearing generation. The two generations are correlated with different conditions of life. The gametophyte is a semi-aquatic plant, a covering of water being a necessity for it to fully consummate its life; on the other hand, the sporophyte is distinctly sub-aerial, dryness being necessary for the proper dispersal of its spores. It is considered that the gametophyte is the older generation, and that the alternation of generations has arisen by the interpolation of the sporophyte generation between pre-existent, subaqueous gametophytes.

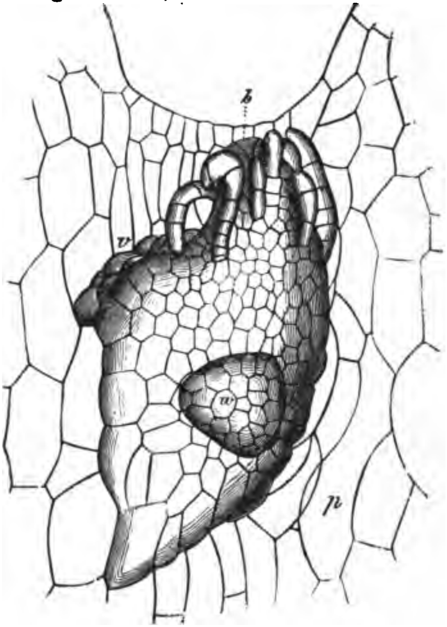

FIG. 89.—Apogamous shoot of *Pteris serrulata*. *p*, prothallium; *b*, first leaf; *v*, apex of stem; *w*, rudiments of first endogenous root. (De Bary.)

The succession, then, is as follows:—**Oospore, Sporophyte, Spore, Gametophyte**, and in *Aspidium Filix-mas* these occur in regular and unbroken order. Ferns are known, however, whose sporophyte is produced directly from the oophyte without the intervention of an oosphere. This phenomenon is known as **Apogamy**, and it occurs in, amongst others, a garden variety of our type fern, viz. var. *cristatum*. Its prothallium produces no archegonia, and the sporophyte arises from it as a shoot. The suppression of the spore is also known to occur in some cultivated varieties of ferns. In *Athyrium Filix-femina*, var. *clarissima*, sporangia are arrested in their development as sporangia, and are converted into prothallia, bearing sexual organs. In *Polystichum angulare*, var. *pulcherrima*, prothallia are produced by vegetative growth from the pinnæ without any connection with sporangia. The suppression of the spore is known as **Apospory.**

CHAPTER VIII.

THE WHITE DEAD NETTLE (*LAMIUM ALBUM*).

EXAMINE a well-grown plant, and note that it is erect, symmetrically grown, well balanced, flexible and elastic, so that it gives to the wind or other pressure, and readily recovers its erect position. The stem is square, with furrowed sides and slightly projecting angles, green and hairy, the nodes swollen, the lower internodes long, the upper short, being regularly smaller and shorter from below upwards; at the apex the immature leaves and growing stem form an apical bud. The leaves arise at the nodes in pairs, opposite to each other, and the vertical plane which passes through the insertions of a pair of leaves at any one node is at right angles to that which passes through the insertions of the pairs of leaves at the nodes immediately above and below. The petioles of the leaves are regularly shorter from below upwards, so that the sub-aerial portion of the plant is pyramidal in shape.

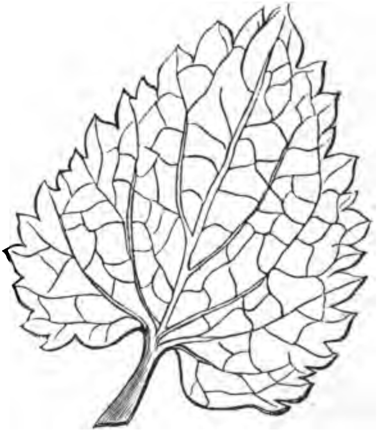

FIG. 90.—Leaf of *Lamium album*; front view.

Each petiole makes an acute angle with the stem; it is deeply channelled on its upper (ventral) surface, with projecting wings, and its lowest portion is almost the width of the stem itself. At its distal end it bears a horizontally disposed entire lamina, with a mid-rib projecting below the lower (dorsal) surface of the leaf, and which is an evident prolongation of the petiole. The lamina is green, hairy, wrinkled, with heart-shaped base, and toothed margin. The lower leaves have rounded, the upper pointed apices. The varying lengths of the petioles, together with the opposite arrangement of leaves on the stem, secure that each lamina receives the maximum of light, and is as little shaded as possible by the leaves above it. The branches of the mid-rib, which run through the whole of the lamina and cause its wrinkled appearance, are removed to some considerable distance below the lower (dorsal) surface of the leaf. In the axils of the leaves arise branches similar in all respects to the main stem. There is the rudiment of a branch in every leaf-axil, but only a very few of them develop.

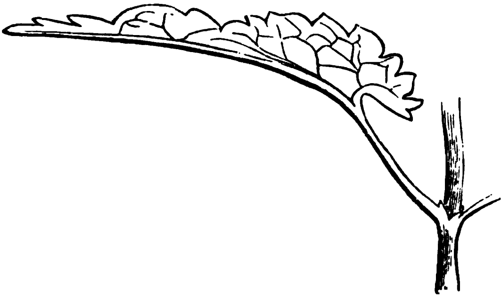

FIG. 91.—Side view of a leaf of *Lamium album*.

The sexual reproductive organs of the plant are borne in conspicuous flowers which grow in the axils of the leaves of the upper portion of the stem, several in each axil. The flower is symmetrical about the antero-posterior median vertical plane. Each flower consists of an outer, green, five-pointed cup, the **Calyx**, formed of five coherent **Sepals** (it is on that account **Gamosepalous**); within this, but projecting considerably above it, is the white, hairy, **Bilabiate** (two-lipped) **Corolla.** The opened mouths of all corollæ are turned outwards. The corolla is formed of five coherent **Petals** (on that account **Gamopetalous**), one forming the lower lip, two the hood, and the apices of two lateral petals being represented by minute teeth. Inside the corolla are four **Stamens**, two longer and two shorter (on that account **Didynamous**), each consisting of a long **Filament**, bearing at its apex an **Anther.** Some distance down the corolla-tube the filaments are adherent to the corolla (and are therefore said to be **Epipetalous**), but they can in the very young flower be traced to their origin on the pad of tissue at the apex of the flower-stem from which all its parts spring. At the bottom of the corolla-tube, and occupying the centre of the flower, is the **Ovary,** from the middle of which arises the long **Style,** which is forked at its apex and terminated by **Stigmas.** The filaments bear the anthers under the hood of the corolla in two pairs, and one of the stigmas projects slightly from between them. The stamens collectively constitute the **Andrœcium,** and from them are developed the male organs of the plant; the ovary contains **Ovules,** and forms, with its receptive apparatus, the stigma, and conducting apparatus, the style, the **Gynœcium,** or female organ. The flower fades, but if fertilization has been effected the ovary grows largely, and divides into four nutlets, which form the fruit of the plant, and from them other plants of the same kind arise under favourable circumstances.

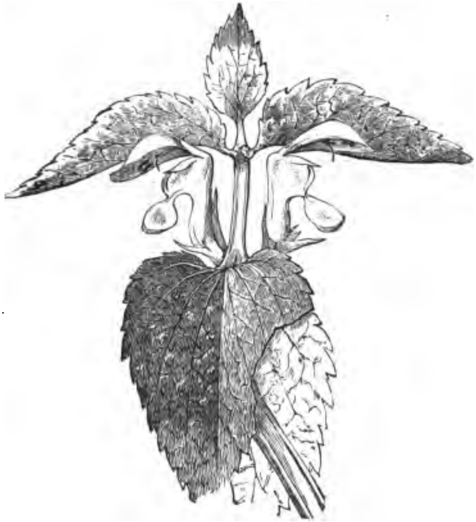

FIG. 92.—*Lamium album;* side view of the flower. A portion of the calyx has been cut away to show the "throat" of the corolla.

A young plant will be found to have a single root, which is a direct continuation of the stem, and from which lateral rootlets arise. The older plant branches copiously at the surface of the soil, the branches running along close to or just below the surface for two or three internodes, sending out roots from the subterranean nodes (the stem is **decumbent**), and then rising erect into the air. The woolly appearance of the young roots, due to the copious production of root-hairs just above the root apex, is easily seen.

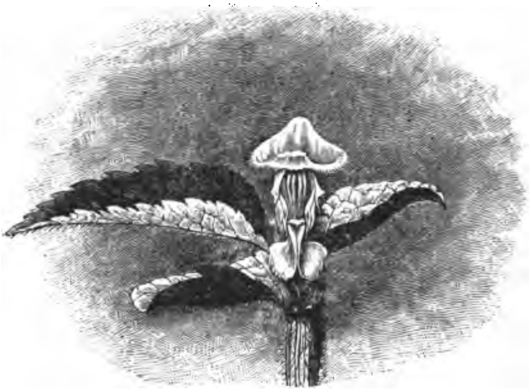

FIG. 93.—Flower of *Lamium album;* front view.

The plant flowers freely throughout the year and very late into the autumn. The severe weather of winter causes it to die down to the creeping stock, from which branches arise in the succeeding spring. It is therefore perennial. The whole plant has a peculiar scent, which can scarcely be described as pleasant.

Practical Work.

Remove a well-grown plant, and note the points already dealt with.

1. Cut across an internode high up the stem, examine the cut edge with a lens if necessary, and note the square section, the projecting angles, and the two furrowed and two rounded sides. Mark the cut edges of eight **Vascular Bundles**, four large ones at the angles, and four smaller ones opposite

the middle of the sides. Each large bundle in the angle is in reality two bundles close together. The bundles lie in a colourless ground-tissue, that portion outside the ring of bundles being known as the **Cortex,** that inside as the **Pith,** and the portions between the bundles as the **Primary Medullary Rays.** The projecting angles of the stem can be easily pulled off through an entire internode. They are formed of a tough, elastic and stringy tissue.

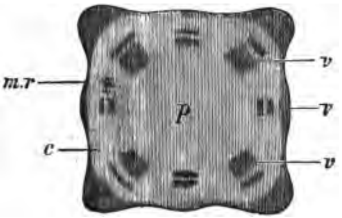

FIG. 94.—Transverse section through young stem of *Lamium album*. *v*, vascular bundle; *c*, cortex; *p*, pith; *m.r*, medullary ray.

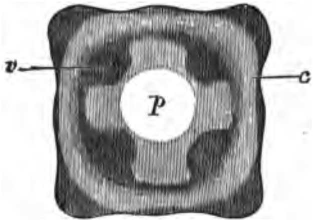

FIG. 95.—Transverse section through old internode of *Lamium album*. *p*, pith cavity; *v*, vascular bundle; *c*, cortex.

2. Cut across one of the older internodes, and note that the bundles are connected together by a ring of tissue which is well differentiated from the ground-tissue. The internode is here hollow, the torn fragments of pith showing clearly how the hollow has been formed. Nevertheless, the hollow of each internode is separated from that of the next by a layer at the node.

3. Take a fresh stem and with a blunt knife remove the cortex, laying bare the vascular bundles throughout the whole length of an internode. They appear as white strands, the white appearance being due to the fact that the vessels of the woody portion are filled with air. The bundles do not anastomose within the internode.

4. Trace the bundles up and down to the nodes, laying bare those which enter the leaf and those in the node on the side adjacent to it.

The leaf-trace consists of three vascular bundles, a median small one (*a*), and on each side of this a larger lateral bundle (*b* and *c*). Immediately on entering the petiole each of the latter gives off a small branch (*d* and *e*) from its outer side, which passes up in the wings of the petiole. The median bundle of the leaf-trace passes down through two internodes of the stem. At the first node below, where there is no leaf on its side of the stem, it anastomoses by short transverse trunks with the bundles in the angles of the stem right and left of it, at the second node it forks and its shanks there fuse with the same two bundles. The larger lateral bundles (*b* and *c*) of the leaf-trace also pass down through two internodes, in the first side by side with a lateral bundle of the next higher leaf, which fuses with it at the first node below, and in the second side by side with a lateral bundle from the next lower leaf, with which it fuses in the second node below. The two bundles running side by side in each angle of the stem are so intimately in contact that they appear like a single bundle.

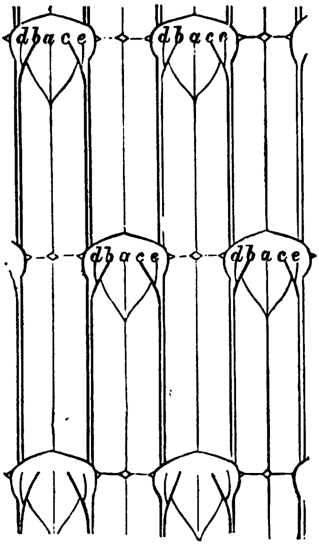

FIG. 96.—The bundle system of the stem of *Lamium album* projected on a vertical plane.

5. Trace up the bundles in the petiole of a leaf by removing tissue from the under side of the petiole until the bundles are exposed.

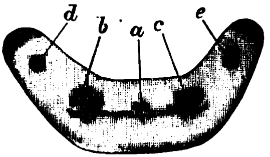

FIG. 97.—Transverse section of petiole of *Lamium album*. *a*, median; *b*, *c*, lateral bundles; *d*, *e*, wing bundles.

Note that ridges on the dorsal side of the petiole correspond with the courses of the large lateral bundles. The small median bundle joins a branch of one of the lateral bundles just after entering the lamina, and the small bundles in the wings of the petiole do the same. The large lateral bundles divide up in the lamina, and their branches radiate to all parts of the leaf and, by frequent anastomoses, form the network of bundles. Their ultimate branches terminate in the teeth of the leaf, and a hand lens will show that the apex of each tooth is a swollen papilla.

6. Dissect out the bundles entering a branch.

Two enter it, one from each adjacent angle of the stem. Each bundle divides into two on entering the branch, and thus form the four bundles in the angles of the stem.

7. Cut across a vigorous root, and note that it possesses a single axial strand, apparently similar to that in the root of Aspidium.

8. Mark the origin of adventitious roots. Usually two are produced at a node, one on each side of a branch in the axil of an abortive leaf.

Microscopical Work.

9. Cut thin transverse sections of an internode not far removed from the apical bud. Mount one in solution of iodine in potassium iodide and examine.

Note that some little distance inside the epidermis there is a ring of cells containing abundant starch-grains. This ring of cells is the **Endodermis.** It is the innermost layer of the **Cortex,** which it thus separates from the **Central Cylinder** of the stem.

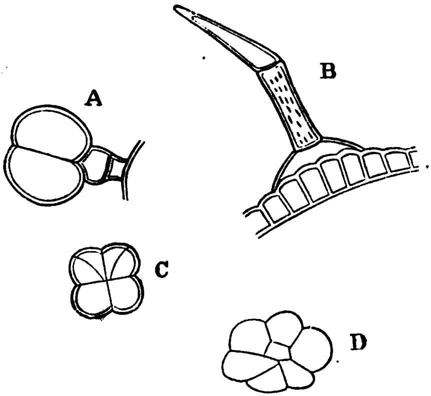

FIG. 98.—Hairs of *Lamium album*. A, glandular; C, surface view of glandular head; B, jointed; D, cells at the base of a jointed hair.

10. Clear similar sections in eau de Javelle, mount in glycerine, examine with a high power, and note the following in order, from without inwards;—

I. *Cortical Tissues.*

(*a*) A single layer of **Epidermal** cells, with thickened outer and inner and thinner radial walls. Single cells grow out into **Glandular Hairs**, each hair consisting of one or two stalk cells and a globular head divided into from two up to eight cells. Long **Jointed Hairs** are also present, chiefly at the angles of the stem. Each of these is seated on several—usually seven or eight—radially-arranged epidermal cells, and consists of two or sometimes three elongated cells marked on the outside with small projections, the uppermost being pointed and the lowest with a bulbous base, the whole standing on a warty elevation. All parts of the plant are covered with one or other of these forms of hairs. Stomata are fairly numerous on the stem.

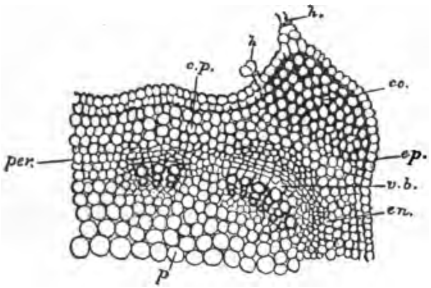

FIG. 99.—Transverse section of a portion of the stem of *Lamium album*. *h*, hairs; *ep*, epidermis; *co*, collenchyma; *c.p*, cortical parenchyma; *en*, endodermis; *per*, pericycle; *v.b*, vascular bundle; *p*, pith.

(*b*) Cells with walls thickened at the angles. This **Collenchymatous** tissue forms a single layer next the epidermis in all parts but the angles, which it fills many layers deep. The cells contain chlorophyll.

(*c*) **Cortical Parenchyma**, consisting of rounded cells. This forms the main mass of the cortex, and contains many intercellular spaces.

(*d*) The **Endodermis**, which is the innermost layer of the cortex. Its cells are similar in size and outline to the cells of the cortical parenchyma, but are distinguishable by their starchy contents. Later, their radial walls are cuticularized.

II. *Tissues of the Central Cylinder.*

(*a*) The **Pericycle.** This forms a complete ring immediately underneath the endodermis. Its cells are smaller than those of the pith or cortex, and are angular, without intercellular spaces. In the very young internode there is but a single layer of pericyclic cells, in a slightly older one two, or in places three. The cells of the pericycle are densely filled with granular protoplasm. Adventitious roots have their origin in this layer.

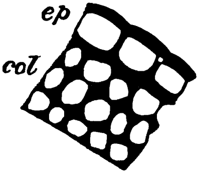

FIG. 100.—Transverse section through *ep*, epidermis, and *col*, collenchyma, of the stem of *Lamium album*.

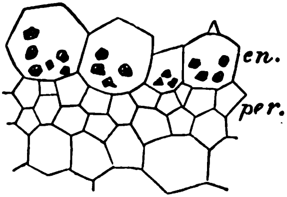

FIG. 101.—Transverse section through endodermis (*en*) and (*per*) pericycle of the stem of *Lamium album*.

(*b*) The **Medulla**, or **Pith**, consisting of larger rounded cells with intercellular spaces. It extends to the centre of the stem.

(*c*) The **Vascular Bundles.** The number will vary according to the age of the internode. In such as that now under examination eight will appear—four large double bundles at the angles and four small ones alternating with them. There is an obvious distinction between the **Xylem** on the inside, the **Phloëm** on the outside, and the separating line of **Cambium.** The bundle is thus not of the concentric type, as in the fern, where the bast surrounds the wood, but is of the **Collateral type.** Note the following:—

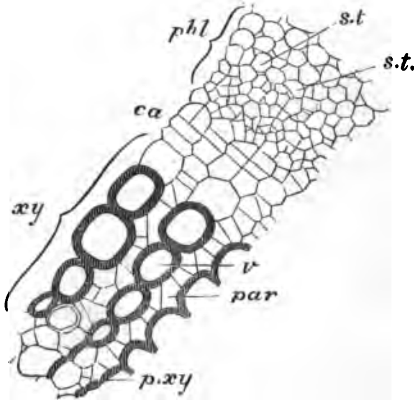

FIG. 102.—Transverse section of a portion of a vascular bundle of *Lamium album*. *xy*, xylem; *phl*, phloëm; *ca*, cambium; *p.xy*, primary xylem; *v*, vessel; *par*, parenchyma of the wood; *s.t*, sieve-tubes.

i. The large vessels of the **Xylem** in radial rows. The smallest, which are also the oldest, towards the centre, where they are early crushed by the lateral growth of the cells on each side, the youngest and largest towards the periphery. The rows are in places duplicated. The thin-walled angular elements between the vessels are parenchymatous cells of the wood.

ii. The **Cambium**, between xylem and phloëm, consisting of very thin-walled cells actively dividing by tangential, and occasionally radial walls. The new tangential walls are formed in the layer close to the phloëm, and the daughter-cells of each cambium cell lie in a radial row, the inner ones forming xylem elements, the outer phloëm. The latter is very little added to by the activity of the cambium, the former very much. Each daughter-cell gradually alters its character, being formed into a permanent member of the wood, or bast.

iii. The **Phloëm**, which consists of **Sieve-tubes**, whose transverse sieve-plates are not unfrequently visible; **Companion Cells**, whose small cross-sections are well seen lying close to the sieve-tubes; and **Bast Parenchyma.**

The section of a very young internode will show but four vascular bundles, two on each convex side of the stem. These are the leaf-trace bundles of the next higher pair of leaves. In an older internode there will be found eight bundles, two in each angle. Later still the small bundles between the bundles at the angles appear in the pericycle.

11. Treat such a cleared section with acid aniline chloride, and note that the walls of the older vessels are lignified. There are, however, full-sized vessels close to the cambium layer which are as yet thin-walled and unlignified.

Schulze's fluid will show that all the elements of the section, with the exception of the xylem vessels, cuticle, and epidermal outgrowths, are of cellulose.

12. Treat with strong sulphuric acid, and note that the cuticle and hairs are not dissolved, and are therefore cuticularized.

13. Treat an uncleared section with solution of iodine in potassium iodide.

Note that the cambium cells and the cells of the pericycle are full of protoplasm. The parenchymatous cells of the cortex and pith also contain protoplasm, as well as the epidermal cells and those of the collenchyma. The mature vessels of the wood are empty; those still immature contain protoplasmic contents. The starch of the endodermis has already been noticed.

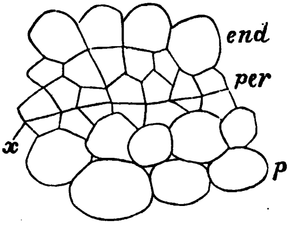

FIG. 103.—Formation of interfascicular cambium from the pericycle of the stem of *Lamium album*. The first tangential walls are formed. *end*, endodermis; *per*, pericycle; *p*, pith; *x*, young wall.

14. Cut sections through a somewhat older internode, clear, and mark that changes have taken place in the pericycle between the primary bundles. In the outer layer of cells, here and there, are formed small groups of phloëm elements, and occasionally these are subtended by xylem vessels before other changes occur in the inner pericyclic layer. Eventually tangential walls in the inner pericyclic layer appear over all the interfascicular portion, and generative cambium is formed which is connected laterally with the fascicular cambium, and so completes the cambium ring. As a necessary result the interfascicular pericycle disappears as such, but persists outside the primary bundles.

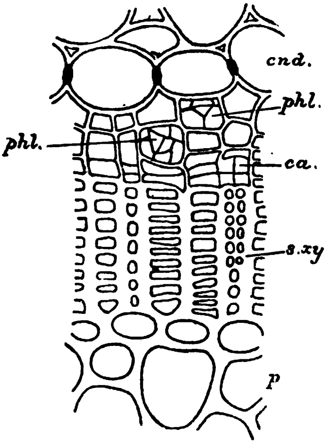

FIG. 104.—Secondary growth in thickness of the stem of *Lamium album*. *end*, endodermis; *phl*, phloëm; *ca*, cambium; *s.xy*, secondary xylem (tracheides); *p*, pith.

15. Cut transverse sections through old internodes near the base of the stem. Clear and examine.

The activity of the cambium has added a thick ring of wood to the stem. The new wood, both in the primary vascular bundles and between them, consists mainly of thick-walled lignified **Tracheides** lying in radial rows. The amount of secondary phloëm formed is extremely small. Note the cavity in the centre of the internode and the manner of its formation.

Since the stem of this plant does not persist for more than one year, the activity of its cambium is necessarily limited. In many other dicotyledons the generative activity of the cambium, suspended in winter, is resumed the following spring. And since, as a rule, the secondary woody elements of spring are much larger than those formed in the previous late autumn, an obvious differentiation of the wood into annual rings is brought about.

The vascular bundles of monocotyledons have no cambium. They have, therefore, no power of forming secondary elements, and are known as **Closed Bundles.** Nevertheless the persistent stems of some arborescent perennial monocotyledons, such as *Ruscus*, *Yucca*, *Dracæna*, and others increase their vascular tissues in this way. By the generative activity of their pericycle are produced secondary, tertiary, etc., parenchyma, in which appear isolated closed collateral bundles, the youngest bundles being towards the periphery of the continually enlarging stem.

The cortical tissues of the stem and root must accommodate themselves to the increasing diameter of the central cylinder. In *Lamium* this increase is mainly in the wood, and although new wood elements are formed centrifugally, the effect is chiefly to push the older elements towards the centre of the hollow internode. What little increase there is in the diameter of the stem and root is accommodated in the cortex by the formation of a few radial walls. In stems which continually increase in diameter this is not enough, and as a consequence cortex and epidermis are ruptured by the growth in thickness of the central cylinder. In such cases the necessary protection to the all-important conducting vascular tissue is given by the production of **Cork.** A ring of cells of the cortical parenchyma at various depths below the epidermis in various plants, or in many cases a layer of cells of the pericycle, becomes meristematic, and forms a **Cork-cambium**, or **Phellogen**, from which by tangential walls are cut off on the outer side cork-cells in radial rows, and, in some cases, on the inner side parenchymatous cells containing chlorophyll. The tissues outside the cork are thrown off, and the cells of the latter being strongly cuticularized afford an efficient protection to the central cylinder. Where the cork is formed from a layer of the cortex near the epidermis, a **Lenticel** is also formed underneath each stoma, through which the intercellular spaces of the stem are kept in communication with the external air. In the elder, for example, by divisions in various planes the cells surrounding the respiratory cavity beneath a stoma form a loose mass of rounded colourless **Packing Cells** which, increasing rapidly in number, rupture the epidermis. The outer packing cells are being continually thrown off and replaced by new cells from within.

16. Cut longitudinal sections of not too young an internode as nearly as possible radially through one of the large bundles at the angles. Clear some in eau de Javelle, examine all with a high power, and note from the outside inwards.

The cells of the epidermis are short, those of the collenchyma much longer, and are obviously derived from mother-cells with pointed ends. They are **chambered** by horizontal walls. The thin-walled cells of the parenchyma of cortex and

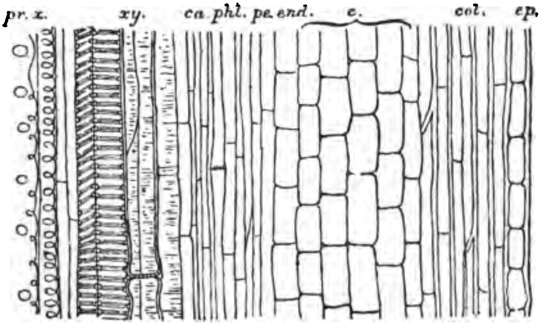

FIG. 105.—Radial longitudinal section through the stem of *Lamium album*. *ep*, epidermis; *col*, collenchyma; *c*, cortex; *end*, endodermis; *pe*, pericycle; *phl*, phloëm; *ca*, cambium; *xy*, xylem; *pr.x*, primary xylem.

pith are not much longer than broad. The cells of the endodermis are clearly recognizable by their contents of starch-grains. The pericycle cells, which come next, are full of protoplasm, and have large and conspicuous nuclei. Note the elements of the vascular bundle in order. The **Sieve-tubes** are long, thin-walled, with horizontal or slightly oblique partitions, which are pierced in the manner already described. The tube is slightly swollen at the sieve-plate, and a plug of highly refractive slime usually covers one side of the plate. The companion cells, smaller than the sieve-tubes, may also be seen. The sieve-tubes can be especially well seen in a longitudinal section taken shortly behind the apex, where for a time they are the only constituents of the bundles. The parenchymatous cells of the phloëm are very evident.

Within the phloëm are the **Cambium Cells**, delicately thin-walled with horizontal transverse walls.

The **Vessels of the Wood.** The oldest are small spiral and annular vessels with a steep open spiral, or widely separated rings. Occasionally at the end of the section a thickening spiral has parted company with its vessel, and it can then be seen how thin is the wall of the vessel around which the spiral has been coiled to strengthen it. Larger spiral and annular vessels succeed with closer thickening bands. The newest formed are comparatively short tracheides, whose longitudinal walls are marked with horizontal bordered pits. Their dividing walls are horizontal, and are not pierced but pitted.

The parenchymatous cells of the wood. These are thin-walled and lie along and between the vessels.

17. Prepare similar longitudinal sections through an old internode. Note here particularly the thick-walled sclerenchymatous tracheides of the secondary wood. Their walls are sparingly pitted with the oblique narrow pits described in the fern.

18. Prepare longitudinal sections through the secondary interfascicular wood, and note that it is composed of tracheides similar to those of the bundles.

19. Prepare tangential sections of the outside of the stem and clear. Note the stomata, which are usually in small groups,

and mark especially the large air-spaces in the hypodermal layer beneath the groups of stomata which send radial branches into the tissue of the cortex, and are therefore best seen in a tangential section. The cells of the hypodermal layer in the neighbourhood of stomata are smaller than at other points.

20. Make tangential sections through the phloëm of a large vascular bundle of an oldish internode. Alcohol material should be used. Clear some and mount others in glycerine without clearing. Examine with a high power.

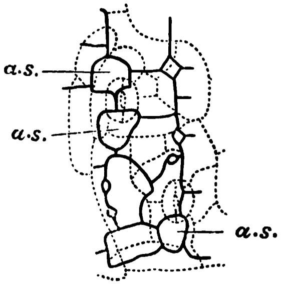

FIG. 106.—Tangential section through the sub-epidermal tissue of the stem of *Lamium album*. The epidermal cells and stomata are outlined by dots. *a.s*, air species.

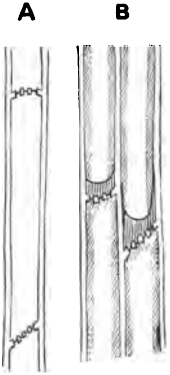

FIG. 107.—Sieve-tubes from stem of *Lamium album*. The contents have been removed from A.

In the cleared sections the sieve-tubes will be found with perforated transverse walls. These are the sieve-plates. In the uncleared sections each sieve-plate will most probably have a plug of a highly refractive colourless substance on its under side. This may either be concave, plane, or convex towards the cavity of the member of the tube. In each case its periphery will be found to be in connection with the contents of the member. If an uncleared section be treated with solution of iodine, the plug as well as the other contents of the tube will show the reactions of protoplasm. Starch-grains may be found in the sieve-tubes, especially about the sieve-plates. Some sieve-plates are to be found on the lateral walls of the tubes where they border other sieve-tubes, and these are also covered on one side with plugs of slimy protoplasm. In all cases these plugs send blunt processes through the pores of the sieve-plates.

Towards the end of the period of flowering the sieve-plates of old tubes are covered with a mass of **Callus**, which completely closes them. Corallin stains the callus a rose-red, and Schulze's fluid brown.

21. Cut longitudinal and transverse sections through a petiole near its base. Clear and examine.

The epidermis and hypodermal layer are similar to those of the stem. The angles are occupied with collenchyma, which is continuous with that of the stem. There are five vascular bundles. The xylem is on the upper, the phloëm on the lower side, so that the bundles pass from stem to petiole without twisting. Each has a layer of cambium, with, however, but a limited power of reproduction. Iodine will show that a starch-bearing endodermis, similar to that in the stem, passes across the petiole on its under side from the outer edge of the bast of one of the large bundles to the outer edge of the bast of the other. A layer of endodermis also lies below the bast of the two bundles in the angles of the petiole. These layers of endodermis are accompanied, as in the stem, by a pericycle which, below the bundles, is several layers thick and collenchymatous.

22. Clear a piece of leaf-blade in eau de Javelle, mount, and examine.

The epidermal cells have sinuous outlines. On the upper side there are no stomata, but pointed and glandular hairs are numerous. Focus below the epidermis, and note the outlines of the palisade cells, which are not everywhere in contact laterally, but show small intercellular spaces. The courses of the anastomosing vascular bundles can be followed. Some terminate in a single spiral tracheide, which ends blindly in the mesophyll. The epidermal cells over the bundles are elongated in the direction of the bundles. On the under side there are numerous stomata, but the directions of the stomatic clefts do not bear any constant relation to the axis of the leaf. The hairs are numerous. Focus below the surface, and notice that the next layer of the mesophyll is looser, with very many and large intercellular spaces.

23. Examine terminal papillæ of the teeth of leaves. The main branches of the vascular bundles converge to, and terminate here. The number of tracheides is largely increased at the end, and they are there associated with a small-celled thin-walled glandular tissue. There are numerous pores and stomata on the upper side of each papilla, and a few towards the apex are larger than the rest and appear permanently open. These are **Water-pores**, and the papilla is a **Water-gland.**

24. Examine fresh leaves mounted in water, and note that the epidermal cells have no chlorophyll corpuscles, but that the guard-cells of the stomata and the underlying mesophyll are abundantly supplied with them. The intercellular spaces of the leaf are filled with air.

25. Cut transverse sections of leaves hardened in alcohol.

Note that the chlorophyll has been dissolved out of the corpuscles. The bundles have no cambium. The wood is on the upper, the bast on the lower side, and collenchymatous pericycle below that. The epidermis shows a well-developed cuticle, and the transverse sections of stomata show that they are in all essential respects similar to those of the stem. The **Palisade** cells are in a single layer, and there are about two layers of irregularly shaped **Spongy Parenchyma** cells, with processes by which they are connected together. There are numerous chlorophyll corpuscles in the cells of the mesophyll.

26. Separate several teeth of the leaf-blade, pack them together all the same way between two pieces of pith, and cut longitudinal sections through the water-glands. Clear some, and examine all with a high power.

If a median section is got it will show the bundles entering the gland and proceeding to its lower side, giving off isolated spiral tracheides, which end blindly in the glandular tissue. This latter is composed of small, thin-walled, nearly isodiametric cells, loosely arranged. There is a large hollow on the upper side of the gland, which is bounded towards the outside by the epidermis and a single layer of irregularly shaped mesophyll cells. There is but an exceedingly thin cuticle on the epidermis over this space, and in the young leaf even this appears to be absent. It communicates with the exterior by pores which are externally similar to but larger than ordinary stomata.

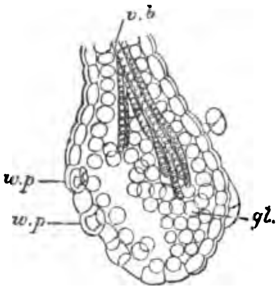

FIG. 108.—Section through a water-gland of *Lamium album*. *v.b*, vascular bundle; *w.p*, water stomata; *gl*, glandular tissue.

27. Select a strong subterranean shoot, wash it well, and harden in alcohol. The apical bud of such a shoot is formed of the growing point enveloped by leaves, which are never developed beyond the initial stages. Being short and comparatively thick, it is more suitable than any other stem apex of the plant for cutting sections. Remove about an eighth of an inch, place between pieces of pith, or imbed in celloidin, and cut longitudinal sections. Clear in eau de Javelle, and examine.

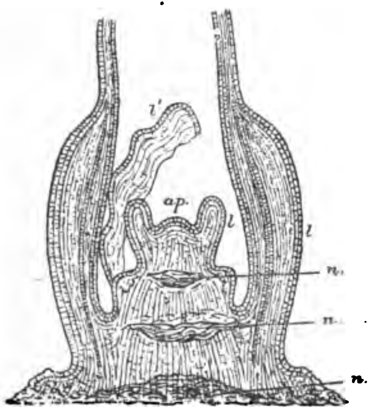

FIG. 109.—Longitudinal section through the apical bud of *Lamium album*. *ap*, apex of stem; *l*, *l*, leaves; *l'*, leaf just below the plane of section; *n*, nodes.

Note the rounded apex, composed of thin-walled meristematic cells, and the leaves originating in pairs behind it. Each leaf appears first as an emergence of the dermatogen and the tissue immediately underlying it just behind the apex. In the axils of the leaves appear the rudiments of buds. At the apex the **Dermatogen**, or formative tissue of the epidermis, is clearly defined, with its periclinal walls thicker than the anticlinal. The cells of this layer form no new periclinal walls. The layer of cells next to the dermatogen is also well defined, with thick periclinal walls. In this layer, from which the collenchymatous envelope of the stem is formed, no new periclinal walls are formed, except in the production of lateral members. For some little distance behind the apex no resolution of the meristem into **Periblem,** or formative tissue of the cortex, and **Plerome,** or formative tissue of the central cylinder, is possible. About the third internode behind the apex, however, strings of elongated cells are seen, which are the unformed elements of the vascular bundles. These are the **Initial Strands,** which are near the dividing lines between plerome and periblem. Very near the apex the smaller, more crowded cells mark out the positions of the nodes.

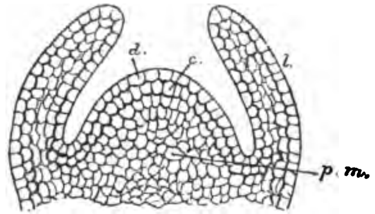

FIG. 110.—Apex of stem of *Lamium album*. *d*, dermatogen; *c*, formative layer of the collenchyma; *p.m*, primary meristem; *l*, young leaf.

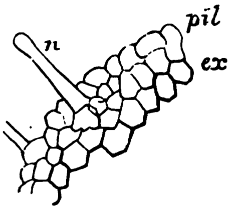

FIG. 111.—Transverse section through a young root of *Lamium album*. *n*, root-hair; *pil*, piliferous layer; *ex*, exodermis.

28. Cut thin transverse sections of a strong young root bearing root-hairs. Alcohol material is best. Clear in potassium hypochlorite solution, and examine first with a low and then with a high power.

Note that there is a **Central Cylinder** surrounded by a **Cortex.** Commencing from the outside, examine the following in order, from without inwards:—

I. *The Cortical Tissues.*

(*a*) The outermost **Piliferous Layer** appearing of crushed and collapsed cells (crushed in cutting), some of which are grown out into unicellular root-hairs.

(*b*) A layer of **Exodermis.** This is the better seen in an older root where its walls do not swell up under the action of the clearing reagent.

(*c*) The parenchymatous cells of the **Cortex** with small intercellular spaces.

(*d*) The **Endodermis,** a single layer of cells without intercellular spaces, each of which shows a dark dot on its radial walls caused by the reflection and refraction of the light away from the surface of its section by these walls, which are puckered from above downwards.

II. *The Tissues of the Central Cylinder.*

(*a*) The **Pericycle,** a layer of small cells which is single in some places and double in others.

(*b*) Several **Xylem** rays alternating with an equal number of **Phloëm** masses. These are on the same circumference, and separated from each other by a small celled tissue. The wood is represented by a few spiral vessels, the smallest and oldest of which are outside; the bast by a few sieve-tubes and parenchyma cells.

(*c*) The **Pith,** a large-celled tissue occupying the centre of the root.

There are no intercellular spaces within the endodermis.

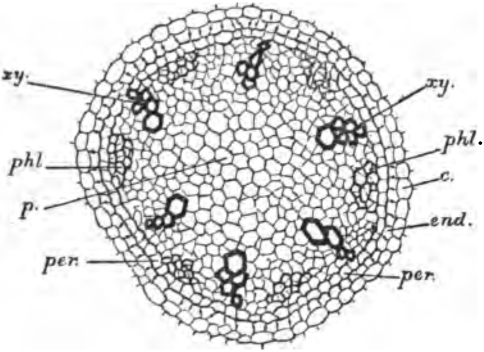

FIG. 112.—Transverse section through the central axis of a young root of *Lamium album*. *c*, cortex; *end*, endodermis; *phl*, phloëm; *xy*, xylem; *per*, pericycle; *p*, pith.

29. Mount a transverse section of the root in strong sulphuric acid, and note that the walls of the cells of the piliferous layer, the anticlinal walls of the endodermis, and in a less degree the walls of the exodermis are undissolved. If the section is tolerably thick, the radial walls of the endodermis float up to the surface and their wavy outline is then seen.

30. Prepare longitudinal sections of this same root. Clear, and examine.

Note that here, as in the fern, the root-hairs are not separated by walls from their mother-cells in the piliferous layer. The only vessels present are spiral. The cells of the piliferous layer and of the exodermis are longer than those immediately within them.

31. Prepare transverse and longitudinal sections through an old root, some distance behind its apex. Clear, and examine.

Note that root-hairs and piliferous layer have all disappeared and the root is bounded by the exodermis. The cells of the cortex are much thicker-walled, and a great many are divided by quite straight, thin, radial partitions. The endodermis is recognizable by its straight, thin, radial walls, which do not now show a dark spot so plainly as before. The pericycle shows thicker walls, but the outer layer of its cells only persists as such. Within this is a broad band of phloëm encircling the xylem, which occupies the whole of the centre of the root, except that a few medullary rays on the same radii as the primary xylem rays penetrate the bast to the pericycle. Sections through roots of various ages show that the secondary thickening of the root has been brought about in this way. The tissue within the phloëm masses becomes converted into cambium, which spreads laterally to the inner layer of pericycle outside the xylem, thus completing the ring, and producing wood on the inside and bast on the outside, except opposite the primary xylem, where parenchymatous cells are formed. The secondary wood formed by this cambium consists mainly of large tracheides with bordered pits. The pith of the young root has become sclerotic by the thickening and lignification of its cell-walls. These walls are all pitted with long oblique pits. The secondary bast consists of sieve-tubes and parenchyma cells.

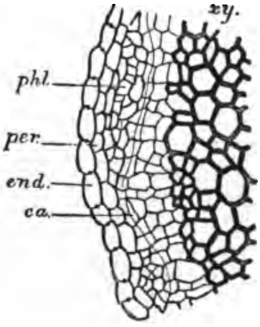

FIG. 113. — Transverse section through a portion of the central cylinder of an older root of *Lamium album*. *end*, endodermis; *per*, pericycle; *phl*, phloëm; *ca*, cambium; *xy*, xylem.

32. In cutting transverse sections of roots, sections will certainly be met with which show the origin of lateral roots. Note that they arise opposite the xylem rays, and that periclinal

walls appear first in the cells of the pericycle. Lateral roots in various stages of development show, however, that the endodermis, cortex, and other tissues take part in their production. The developing root forces its way through the cortex and emerges by rupture of this tissue and those without it. It is, therefore, of **Endogenous** origin.

33. Take strong young adventitious roots, wash, remove a quarter of an inch of root-tip, harden in alcohol, imbed in celloidin on the top of a cork or place in cleft pith, and cut thin longitudinal sections. Select median sections, mount in glycerine, and examine with a high power. Clear others in eau de Javelle, mount, and examine.

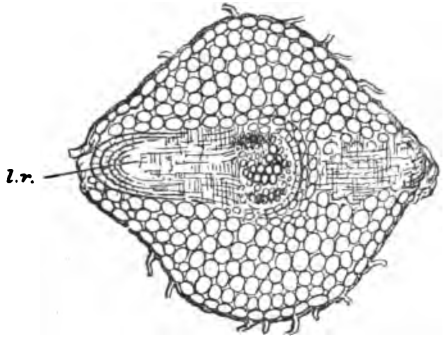

FIG. 114.—Origin of lateral root of *Lamium album*. *l.r*, young lateral root forcing its way through the cortex of the primary root. Another, almost opposite this, lies just below the plane of section.

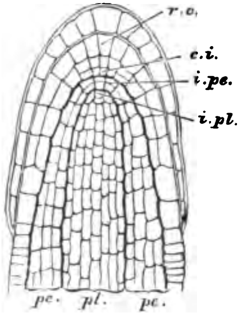

FIG. 115.—Longitudinal section through the root apex of *Lamium album*. *r.c*, root-cap; *pl*, plerome; *pe*, periblem; *i.pl*, initial layer of the plerome; *i.pe*, initial layer of the periblem; *c.i*, common initial layer of the dermatogen and calyptrogen.

The uncleared sections show the **Root-cap** outside, a cylinder of **Plerome** with rounded apex occupying the centre, and between the region at the apex of the plerome and the internal layers of the root-cap a mass of thin-walled cells full of granular protoplasm. This is the **Punctum Vegetationis.** Note the large nuclei of all the meristematic cells. The cleared section shows that at the apex of the plerome is its **Initial Layer**, capping it, and from the cells of which the whole of the plerome is derived. The plerome is the formative tissue of the axile vascular bundles, and its outer layer forms the pericycle. Above the initial layer of the plerome is the initial layer of the **Periblem,** the formative tissue of the cortex. Its inner layer forms the endodermis. Trace back its cells on both sides, and note how longitudinal divisions increase the number of its rows. Above the initial layer of the periblem is the common initial layer of the formative tissue of the piliferous layer and the root-cap. The cells of this layer produce the **Calyptrogen,** or formative tissue of the root-cap by periclinal walls, and the **Dermatogen,** or formative tissue of the piliferous layer by anticlinal walls.

The Flower.

All parts of the flower are developed as emergences of the somewhat broadened pad of tissue, the **Receptacle** or **Torus,** which terminates the very short flower-stalk. The first to appear are the segments of the calyx, which emerge as papillæ around the rim of the receptacle. Subsequent growth of the tissue of the rim between the papillæ produces the calyx-tube. Next the five petals of the corolla appear, also as papillæ, within the ring of sepals and alternating with them. Subsequent growth of the tissue between these papillæ produces the corolla-tube,

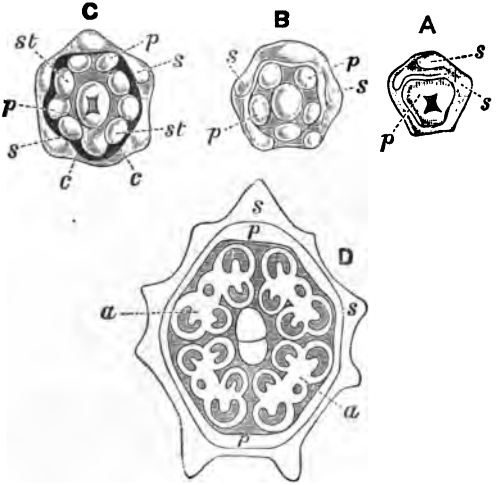

Fig. 116.—Development of the flower of *Lamium album*. A, B, C, successive stages; D, transverse section of flower-bud. *s*, sepals; *p*, petals; *st*, stamens; *c*, carpels; *a*, anthers. (After Sachs.)

Within these appear four projections, alternating with the petals. These are the stamens. No sign of a stamen appears between the two posterior petals. The growth of the tissue between the stamens and the corolla-tube raises the former on the latter and causes the stamens to be epipetalous. The development of the stamens is much more rapid than that of the corolla. The andrœcium is in an advanced stage of development whilst the corolla is still immature. Last to appear is the gynæcium (ovary), which is on the apex of the floral axis and occupies the centre of the flower. The mode of origin of the floral organs on the floral axis is similar in all essential respects to that of leaves on the vegetative axis.

34. Examine the calyx thoroughly, cutting longitudinal and transverse sections.

Note a well-developed epidermis on each side, with numerous stomata. The mesophyll consists entirely of an extremely loose spongy parenchyma. The vascular bundles are well developed, and are devoid of cambium. But their woody elements are strongly thickened, and the cells of the pericycle, forming here and there small strands of phloëm, are also strongly thickened and lignified. They are therefore developed into broad strengthening bands of thick-walled sclerenchymatous cells reaching almost from bundle to bundle. The calyx is thus greatly strengthened.

35. Make a detailed examination of the corolla under the dissecting microscope. Cut sections and mount pieces for surface examination under a high power.

There is a well-developed epidermis. The outside of the hood is closely beset with long-jointed hairs. Glandular hairs are to be found on almost all parts. The epidermal cells on the inside of the tube grow out into conical papillæ, which in some cases attain an immense size. There is a loose mesophyll except in the lower lip. The vascular bundles anastomose as in the leaf, but they are small in the upper portion of the corolla, consisting only of a few spiral vessels accompanied by thin-walled elongated cells. The corolla-tube bulges out in front and forms an evident "throat," which is, in fact, a slightly developed spur. Immediately below this there is a thickening of the tube on the inside, forming a "collar" of tissue thickly covered with long pointed unicellular hairs, turned inwards and completely closing the tube except at the back, where the ring is incomplete. The cells of the very young corolla have chlorophyll corpuscles, and a few stomata are to be seen. The chlorophyll is early lost, and the white colour of the mature corolla is due to the air amongst its cells.

As has been stated above, the stamens arise independently within the corolla-tube, and subsequently become epipetalous by the growth of the tissue between their filaments and the corolla-tube. They are the most rapidly growing of all the floral organs. The protuberance on the receptacle, which is the earliest indication of each, is the anther, which shows two well-shaped lobes whilst the filament is still very short. Later, a rapid elongation of the filaments takes place, so that just before the flower opens they are much bent. At the opening of the flower they quickly straighten themselves, and raise the anthers to their final position beneath the hood. The filament is attached to the top of the anther, whose two lobes are separate and early diverge below. In the open flower failure to straighten out a twist in the top of the filament brings the two anther-lobes in a line with the filament. The outer side of each anther-lobe, as well as the filament, is covered with pointed and glandular hairs.

36. Make longitudinal and transverse sections through a flower-bud of about one-twelfth of an inch in length by placing between pith or imbedding in celloidin. Clear some in eau de Javelle, and compare uncleared sections with these. Examine with a high power sections of anthers.

There is an evident epidermis and cuticle covering the anther. Beneath this the transverse section will show four places where the two layers of cells immediately succeeding the epidermis are larger and more regularly arranged than the deeper lying meristematic cells. If at the right stage the outer of these layers will be dividing by periclinal walls into two layers. Examination of sections cut from slightly older buds will show that the outer of these two layers formed from the original hypodermal layer again divides by periclinal walls into three layers. There are now four layers of cells formed from the original hypodermal layer. Calling them, reckoning from the epidermis, *a*, *b*, *c*, and *d*, and calling the layer which was originally second from the epidermis, but is now fifth, *e*, their fates are as follow :—

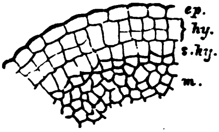

FIG. 117.—Longitudinal section through a very young anther of *Lamium album*. *ep*, epidermis; *hy*, hypodermal layer, *s.hy*, subhypodermal layer; *m*, meristem.

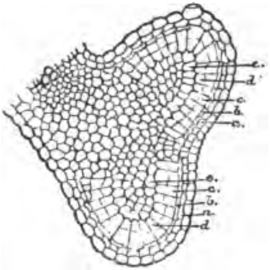

FIG. 118. — Transverse section through a slightly older anther of *Lamium album*. Letters as in the text.

(*a*) Its cells become thickened with U-shaped fibrous bands, the opening of the ⊂ being towards the epidermis. When the pollen-grains are mature this **Fibrous Layer** has a great deal to do with the rupture of the walls of the anther-lobe along its median line.

(*b*) These cells lose their contents, and are finally crushed flat against the fibrous layer by the development of the sporogenous tissue within. This layer has been called the **Transitory Layer.**

(*c*) This is the **Outer Tapetal Layer,** some of whose cells may again divide by periclinal walls. Unlike layers *a* and *b*, its walls remain extremely thin. The contents of its cells are absorbed and its cell-walls broken down by the rapidly growing spore-tissue developed from *d*.

(*e*) This is the **Inner Tapetal Layer.** It has the same function as, but differs in origin from, the outer tapetal layer. The cells of the inner tapetal layer are absorbed before those of the outer. The two layers overlap and meet around *d*, so that a **Loculus** is formed in each half of the anther lobe, and in each loculus the tissue developed from *d* is isolated and freed. Layers *c* and *e* may be together called the **Tapetum.**

(*d*) This layer is the **Archesporium.** Its cells divide in various directions and form the **Pollen-mother-cells.** The nucleus of each pollen-mother-cell divides into two, and these again divide, whilst the cell-wall thickens, and ingrowths from it separate the four nuclei, together with investing cytoplasm, from each other. By this time the pollen-mother-cells are separate from each other and free in the loculi of the anthers. The immature pollen-grains are then covered with a special cellulose coat, and the walls of the mother-cells are dissolved and converted into mucilage, in which the pollen-grains float. The latter continue to grow at the expense of this fluid and the cells of the tissue projecting into the loculus from the axis of the anther, and finally fill the cavity with a powdery mass of pollen-grains.

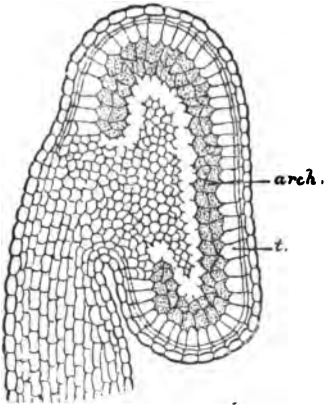

FIG. 119.—Longitudinal section through a still older anther-lobe. The inner tapetal layer has disappeared. *t*, tapetum; *arch*, archesporium.

37. Open a bud about one-eighth of an inch in length, and tease out the contents of an anther. Staining is not necessary, although hæmatoxylin brings out the details of the pollen-mother-cells well. Mount in water or glycerine.

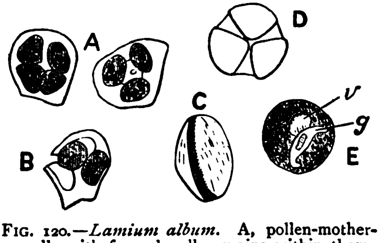

FIG. 120.—*Lamium album.* A, pollen-mother-cells with formed pollen-grains within them; B, two pollen-grains have escaped; C, ripe pollen-grain dry; D, in water; E, pollen-grain in glycerine, showing generative (*g*) and vegetative nuclei (*v*).

Observe the tetrads in the mother-cells. It is not probable that all will be exactly at the same stage of development;

therefore, look out for instances where the nucleus is dividing. Some of the tetrads swell up, burst the wall of the mother-cell, and are so set free.

38. Open a ripe anther, and examine the pollen (dry) with a high power.

Each grain is a triangular prism with rounded ends. There are faint markings on its outer walls.

39. Mount ripe pollen-grains in water, and examine. The grains swell out until they are spherical. It is now seen that each grain has three bands of a yellowish substance on its outside which narrow and meet at the poles and broaden out at the equator. The contents are granular, and with difficulty one and sometimes two nuclei can be seen. The coat between the bands is colourless. Sulphuric acid shows that they are strongly cuticularized. In the dry grain the bands are in contact edge to edge and the colourless portion is folded within.

40. Mount dry pollen-grains, not quite mature, in pure glycerine.

They slowly swell up, and then some of them show a spindle-shaped straight or curved cell with a large nucleus, immersed in the granular cytoplasm of the pollen-grain. This is the **Generative Nucleus.** At an earlier stage it lies in the small cell against the cell-wall, but afterwards separates from it, loses its special wall, and the nucleus is set free. The highly refractive nature of its contents enables the generative nucleus to be easily seen. There is another nucleus in the grain, however, the **Vegetative Nucleus,** which can be made out by careful focussing and manipulation of the light. It is oval and smaller than the generative nucleus.

41. Mount ripe pollen-grains in a drop of weak solution of sugar in a moist cell. Keep in the dark for twenty-four hours.

Most of the grains will have protruded a **Pollen-tube** at one of the thin equatorial regions, with dense protoplasmic contents, in which nuclei can be distinguished after careful staining.

Before the homologies of the flowering plants with the ferns were known or suspected, the names stamen, anther, pollen, etc., were given to the structures just described, and for convenience they are still retained. It must not be forgotten, however, that the stamen is a **Sporophyll**, the loculus of the anther is a **Microsporangium**, and the pollen-grains are **Microspores.** The division of the microspore into two cells is the formation of a rudimentary **Prothallium**, the generative cell representing an **Antheridium.**

42. Cut transverse sections of a ripe anther immediately before dehiscence.

Note that the septum separating the two loculi of each anther-lobe is completely absorbed. At its former point of union with the peripheral layers, nothing now bounds the cavity but the single layer of dried epidermis. This is subsequently ruptured by the contraction of the fibrous layer, and in this way dehiscence takes place.

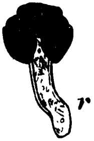

FIG. 121.—Germinating pollen-grain of *Lamium album*. *r*, pollen tube.

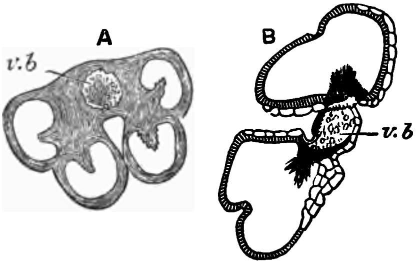

FIG. 122.—Transverse sections of anthers of *Lamium album*. A, at the close of the formation of pollen-grains; B, just before dehiscence; *v.b*, vascular bundle in the connective.

43. Pull out the corolla from a widely opened flower. In the lower end of the corolla-tube beneath the ring of hairs will be found a drop of clear liquid. Suck this out and notice that it is sweet.

44. Dissect away the calyx from the gynæcium. Around the anterior carpel is a ridge of yellow tissue. This is the **Nectary.** An opportunity of seeing its internal structure will be afforded when making sections of the gynæcium.

On account of its small size and somewhat abnormal structure and development, it is better that the student should examine other gynæcia before attempting that of *Lamium album.*

45. Cut transverse sections of the immature gynæcium of *Phaseolus vulgaris* (the French Bean), or of one of the ovaries of *Caltha palustris* (Marsh Marigold). Alcohol material will be best.

The **Carpel,** which constitutes the wall of the ovary, has a structure similar to that of a foliage leaf. There are an inner and an outer epidermis with layers of mesophyll between, and occasionally stomata will be found on it. The mid-rib is evident, and on the opposite side is the **Ventral Suture,** which marks the line of junction of the margins of the carpel and along which the **Ovules** are arranged in two rows, one on each margin of the carpel. The tissue from which the ovules arise is the **Placenta,** and in these plants the **Placentation** is **Sutural.** The ovary is evidently formed of a single carpel folded on itself and enclosing one loculus.

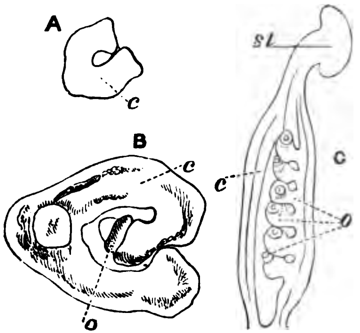

FIG. 123.—A, B, transverse sections through the ovary of *Phaseolus vulgaris* at different ages. C, longitudinal section; *c*, carpel; *st*, stigma; *o*, *o*, ovules. (After Sachs.)

46. Examine in the same way the ovary of *Viola tricolor* (the Pansy), and note that its wall is formed by three carpels uniting at their edges and enclosing a single loculus. Examine also the ovary of one of the Liliaceæ (lily, tulip, hyacinth, etc.). Here also the ovary is formed by three carpels, but their edges are incurved and meet in the centre, and so form a **Trilocular** ovary. In Viola the placentation is **Parietal,** in the Liliaceæ **Axile.** In all these plants the placenta is on the margin of the carpel, and this is the rule.

47. Make transverse sections through one of the ovaries, hardened in alcohol, of *Aconitum napellus* (Monkshood). Select a section which has passed medianly through an

ovule, and examine first with a low, then with a high power.

The ovary is monocarpellary and unilocular, and the placentation sutural.

The ovule has a short stalk, the **Funicle,** the upper part of which is coherent with the ovule along one side. This portion is the **Raphe.** A vascular bundle runs through funicle and raphe. The middle of the ovule is occupied by a small-celled tissue, the **Nucellus.** This is enclosed by an **Outer** and an **Inner Integument** except at one point where a small canal, the **Micropyle,** leads from the loculus of the ovary down to the nucellus. Where the raphe is coherent with the tissue of the nucellus is the **Chalaza.** The outer integument is wanting on the side of the raphe, and because the ovule is so bent on its funicle as to bring the micropyle close to the funicle whilst the nucellus remains straight, it is said to be **Anatropous.** This is the usual form of the ovule. In a few cases it is straight, and is then called **Orthotropous,** whilst in a few others the nucellus is bent, and the name **Campylotropous** is applied to them. One cell of the nucellus is very much larger than the others. This is the embryo-sac, in which will be found naked cells, the products of the division of its nucleus. One of these is the **Oosphere** (egg-cell), which, being fertilized by the contents of a pollen-tube, develops into the embryo.

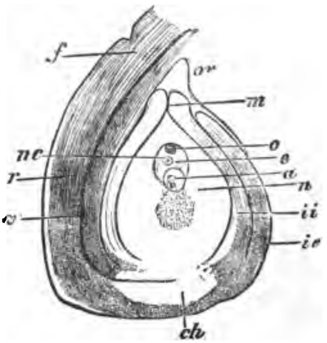

Fig. 124.—*Aconitum napellus.* Median longitudinal section through an ovule. *f*, funicle; *r*, raphe; *v*, vascular bundle; *ie*, outer integument; *ii*, inner integument; *n*, nucellus; *ch*, chalaza; *e*, embryo-sac; *a*, antipodal cells; *o*, oosphere; *nc*, secondary nucleus; *m*, micropyle; *or*, wall of the ovary. (Strasburger.)

To follow out the changes in the embryo-sac, it is best to work with ovules, which are small and transparent, so that the embryo-sac can be examined through the walls of the ovule when the latter is mounted whole. Very suitable material for this purpose is *Monotropa hypopitys* (Yellow Bird's Nest), which grows in woods without undergrowth (fir, beech, birch, etc.), and is not unfrequent in England.

48. Select some flowers just opening and others fading. Open the ovaries, remove ovules, mount in a very weak solution of sugar, and focus for an optical section showing the embryo-sac with a high power.

There is no other representative of the nucellus to be seen than the embryo-sac. At its apex, which lies at the bottom of the micropyle, are three naked cells—two close to the apex and one a little deeper. The two former are the **Synergidæ**, the latter the **Oosphere.** Lying deeper in the embryo-sac is its **Secondary Nucleus** and at its farther end are three cells, the **Antipodal Cells.** Of these seven cells all ultimately

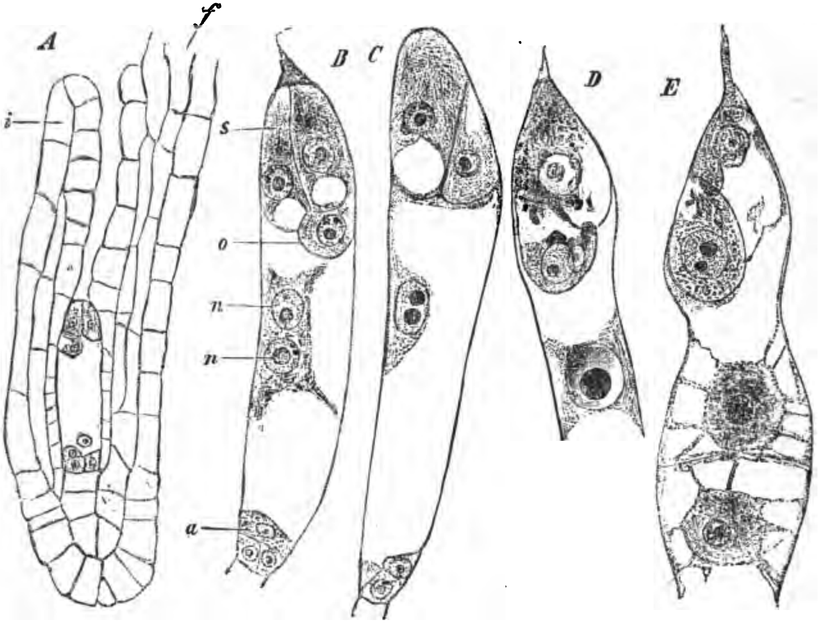

Fig. 125.—*Monotropa hypopitys*. *A*, a mature ovule; *f*, funicle; *i*, integument. *B* and *C*, embryo-sac; *s*, synergidæ; *o*, oosphere; *n*, *n*, formation of secondary nucleus; *a*, antipodal cells. *D* and *E*, upper parts of the embryo-sac. *E* show the first division for the formation of the endosperm. (Strasburger.)

perish except the oosphere, which develops into the **Embryo**, and the secondary nucleus, which produces the **Endosperm.**

On an older ovule which has been fertilized fragments of pollen-tubes may be found attached to the micropyle. The fertilized oosphere, now an **Oospore**, has taken on a delicate cellulose coat and one or both synergidæ are not recognizable. The secondary nucleus divides into two, which are separated by a wall across the embryo-sac. This is the commencement of the formation of the endosperm, which in this and other ovules with small embryo-sacs is formed by the actual division of the sac, but in others is commenced by the division and subdivision of the nucleus, the daughter-nuclei being scattered about in the protoplasm which lines the sac. Later these become coated, and then by their division they fill the embryo-sac with endosperm, in which lies the embryo. The oospore by division forms a cell-row, at the end of which is developed the **Embryo.**

In the typical angiospermous ovule the development of the embryo-sac and its contents is in this manner: A cell immediately underlying the limiting layer of the nucellus at its apex divides into two; the outer further divides and forms a **Tapetum**; the inner is the **Archesporium**, which also further divides into an axile row of cells, the deepest lying cell being the **Embryo-sac.** The nucleus of the embryo-sac divides into two, which retreat to opposite ends of the cell, where each divides, and the daughter-cells again divide, so that four cells are now at each end of the sac. One from each end then advances to the middle of the cell, where they fuse and form the **Secondary Nucleus.** The three at the micropylar end are the **Egg Apparatus**—the **Synergidæ** and **Oosphere**, the three at the other end are **Antipodal Cells.** Although the terms ovule, embryo-sac, etc., are retained as convenient special names for these structures in angiosperms, yet it must be distinctly understood that the ovule is a **Macrosporangium**, the embryo-sac a **Macrospore**, which never leaves its macrosporangium and which produces a rudimentary **Prothallium** and a single **Archegonium** represented by the egg-apparatus.

49. Select a bud of *Lamium album* about one-tenth of an inch in length, harden in alcohol, place inverted between pith or imbed in celloidin, and cut successive transverse sections from its base. Clear in eau de Javelle, and examine them as nearly as possible in the order in which they were cut.

The first few which pass through the flower-stalk may be neglected; soon one will be found which has gone through the base of the ovary. From this and succeeding ones the structure of the ovary may be determined. There are two carpels, with evident mid-ribs, outer and inner epidermis, and layers of mesophyll. Glandular hairs are later borne in small number on the inner epidermis. The two halves of each lamina of the carpels are rolled upon themselves. In the lower part of the ovary the opposing surfaces of opposite carpels cohere, but the halves of the laminæ of the same carpel do not meet in the middle. The mid-ribs project slightly inwards, so that although the ovary is here four-chambered it is yet unilocular. Sections higher up show that the upper part of the half of each lamina is quite separate from its opposite lamina, and from its own mid-rib, whilst the mid-ribs arch over the apex of the stem and meet and fuse in the middle line, forming thus the style. There is one ovule in each chamber of the ovary borne on the carpel near its base. The lower sections show that the nectary is an outgrowth from the base of the anterior carpel.

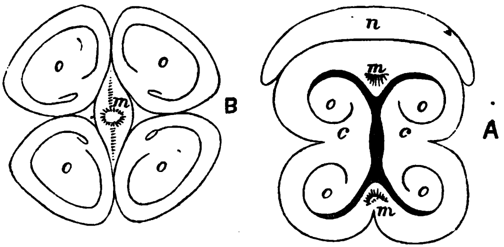

FIG. 126.—Transverse sections through the ovary of *Lamium album*. A passes through the base of the ovary; B, higher up; *n*, nectary; *m*, mid-ribs of the carpels; *c*, *c*, the line along which opposite carpels are connected; *o*, *o*, ovules.

50. Cut longitudinal sections of young flower-buds, com-

mencing with some about one-tenth of an inch in length. Make out from the younger the relations of the parts alluded to in the previous paragraph. In older buds it will be seen to how great an extent the laminæ of the carpels develop upwards to form distinct mericarps, and at the same time the cells of the outer epidermis lengthen greatly from the three-rowed mesophyll outwards, and those at the outer angle divide by periclinal walls. The ovule fills the young ovary, but the latter grows the faster and when the ovule is ready for fertilization there is a large ovarian space above it.

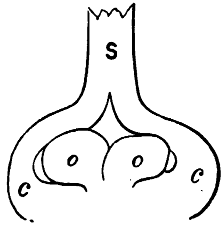

FIG. 127.—Longitudinal section through a very young ovary of *Lamium album*. *c*, *c*, carpels; *o*, *o*, ovules; S, style.

From cleared and uncleared longitudinal sections of all ages trace as far as possible the development of the ovule and its contents. The ovule first appears as a small projection of cellular tissue from the base of the carpel, and is for a time nearly straight. Soon the single integument grows up around the nucellus from its base, quickly overtops it, and grows around its apex, leaving an open micropyle. During this growth the ovule is bent over until the micropyle is brought close to the short funicle. The nucellus is also bent, so that the mature ovule is campylotropous. The funicle is adherent to the carpel right up to the chalaza, and its vascular bundle is composed of phloëm only. In the axile row of cells

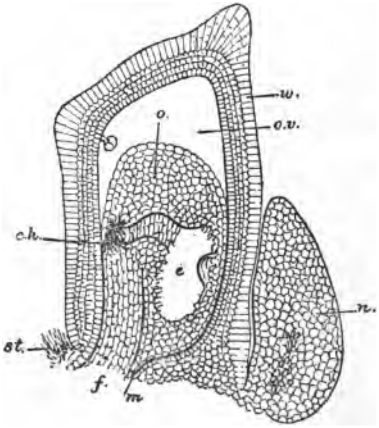

FIG. 128. — Longitudinal section through one chamber of the ovary of *Lamium album*. *n*, nectary; *s.t*, base of style; *w*, wall of ovary; *o.v*, ovary cavity; *o*, ovule; *e*, embryo-sac; *c.h*, chalaza; *f*, funicle; *m*, micropyle. The section has been cleared by eau de Javelle.

of the nucellus an archesporium and tapetal cells are formed, but these changes are not easy to follow in this plant. The embryo-sac grows rapidly and displaces the tissue of the small nucellus. It is bent at right angles and extends from the chalaza to the upper end of the micropyle. It develops some curious vermiform hollow appendages, which are pressed in a confused coil against the wall of the sac in the region opposite the chalaza. If an ovule be removed from the gynæcium of an open flower, mounted in water or glycerine, and sharply pressed under a cover-glass, the embryo-sac is squeezed out and can then be examined. It is best, however, to examine it in carefully made sections.

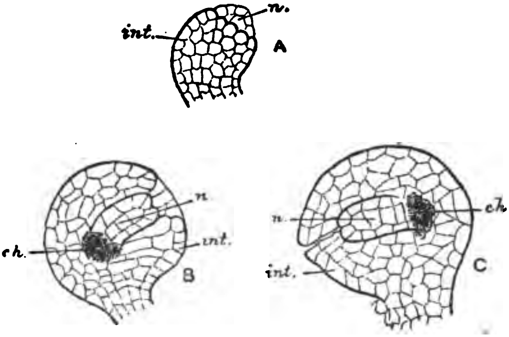

FIG. 129.—A, B, C, successive stages in the development of the ovule of *Lamium album*. *int*, integument; *n*, nucellus; *ch*, chalaza.

The nucleus of the embryo-sac goes through the same divisions as those of the plants already described, and evidences of these changes will be met with in sections. When ready for fertilization there are two synergidæ at the micropylar end, with the oosphere lying somewhat deeper. Deeper still will be found the secondary nucleus, suspended by strands of protoplasm from the wall of the sac. The antipodal cells are clothed before fertilization. One or two may sometimes be found, but since they lie somewhat free they are frequently removed in cutting and mounting the section.

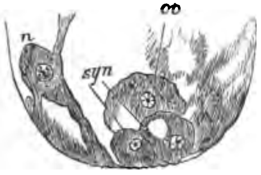

FIG. 130.—Micropylar end of the embryo-sac of *Lamium album* before fertilization. *oo*, oosphere; *syn*, synergidæ; *n*, secondary nucleus.

The **Style**, which by the upward growth of the mericarps of the ovary is attached below to near its base, and is therefore said to be **Gynobasic**, is traversed by the two vascular bundles from the mid-ribs of the carpels. These are quite separate throughout, and each passes into a branch of the forked style. The centre of the style is occupied by the very long narrow thin-walled cells of the **Conducting Tissue.** The **Receptive (Stigmatic) Surfaces** are situated at the ends of the forked style, and are small in extent. The cells here, which are wrinkled and elevated into papillæ, secrete a viscid substance. The pollen-grains, whether falling naturally on the stigma, as they may do, or carried there from other flowers by insects, are retained by the secretion and germinate, sending out pollen-tubes which enter the tissue of the style and grow down through the conducting tissue, being nourished by the contents of its cells.

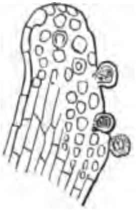

FIG. 131.—Stigma of *Lamium album* with germinating pollen-grains.

Examine the stigma of a fading flower for penetrating pollen-tubes. It will be best to place it momentarily in alcohol before mounting in water or glycerine.

51. Boil the style of a fading flower on the slide in weak glycerine for a moment, cover, and examine.

The cells of the conducting tissue and that lying outside it will thereby be rendered more transparent, and the pollen-tubes, full of densely granular protoplasm, will be brought into view traversing it.

52. If careful longitudinal sections be made of such a style hardened in alcohol—not a difficult operation if it be cut between pith or imbedded in celloidin—the pollen-tubes with their highly refractive contents can be seen traversing the long, delicately walled cells of the conducting tissue.

Transverse sections of the style may be cut by packing several between pieces of pith. Alcohol material must be used and the sections mounted in alcohol or glycerine. The conducting tissue is then seen to form a central cylinder of very small cells which lie in a mucilage derived from their outer walls. Immersion in water will dissolve the mucilage and isolate the delicately walled cells. A solution of corallin in sodium carbonate stains the mucilaginous walls pink.

The pollen-tubes enter the cavity of the ovary and, bending around the funicles, enter the micropyle and ascend it to the embryo-sac. The micropyle lies so close to the funicle and the bottom of the ovarian loculus that it is difficult to see this in position. If, however, a fertilized ovule be removed, fragments of the torn pollen-tube can be seen attached to the micropyle. There is no doubt that in Lamium the details of fertilization are similar to those in plants which have been directly observed during fertilization, viz. the generative nucleus of the pollen-grain which is borne near the apex of the pollen-tube is led by the synergidæ to the oosphere, with whose nucleus it fuses. The synergidæ perish in the process, the oosphere as a first result takes on a cellulose coat. Meanwhile the embryo-sac at its angle commences to make a large upward growth into the thick integument, hollowing out a chamber in which the embryo is to lie. Ovary and ovule also grow rapidly upward, the limiting layer of cells of the latter quite altering their character. The changes in the ovule and its contents which result from fertilization may conveniently be taken separately.

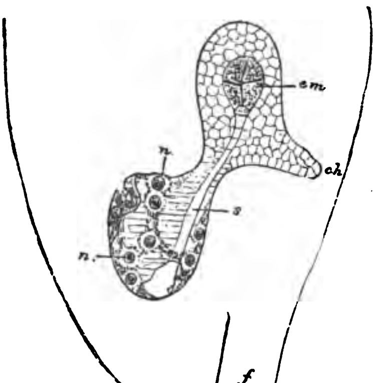

FIG. 132.—Development of the embryo and suspensor of *Lamium album*. *f*, funicle; *ch*, chalaza; *m*, micropylar end of ovule; *s*, suspensor; *em*, embryo; *n*, nuclei in the lower end of the embryo-sac.

The **Oospore** secretes a cellulose coat and attaches itself to the wall of the embryo-sac at the micropylar end. It then divides repeatedly into a string of cells, the **Pro-embryo**, which pushes its apex up into the newly enlarged portion of the embryo-sac. The swollen end-cell of the pro-embryo now divides by three walls at right angles to each other into octants. These with the next cell, which arches upwards, form the **Embryo**; the rest of the string of cells is the **Suspensor**, which eventually perishes. Periclinal walls now appear in the embryo, and further growth and division of cells follow. The embryo elongates and lateral outgrowths form the two **Cotyledons.** The mature embryo as it lies in the ripe seed consists of a straight axis, whose lower root end — the **Radicle** — is turned towards the micropyle, and upper end—the **Plumule**—towards the top of the ovule, and two **Cotyledons** which are attached at the same level to the axis close to the plumule, so that the epicotyledonary portion of the axis—the **Epicotyl** — is very short, and the **Hypocotyl** comparatively long. The distinctive formative layers of the tissues are already present in the embryo.

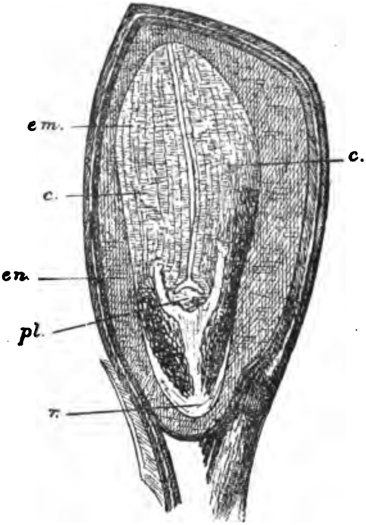

FIG. 133.—Longitudinal section through a mericarp of *Lamium album*. *em*, embryo; *en*, endosperm; *r*, radicle; *pl*, plumule; *c*, *c*, cotyledons.

The **Secondary Nucleus** of the embryo-sac, with a portion of protoplasm aggregated around it and constituting it a naked cell, divides and subdivides, the daughter-cells lying naked in the protoplasm lining the embryo-sac. In the upper chamber of the sac containing the embryo these naked cells become coated with cell-walls, and the formation of cells spreads by division until the whole of the embryo-chamber is full of the thin-walled cells of the **Endosperm** in which the embryo lies. The lower chamber of the sac, which at first contains a few naked cells derived from the secondary nucleus, is finally obliterated by the rapid growth of the embryo-chamber and its endosperm, and its naked cells soon perish and are absorbed. Meanwhile the embryo-sac with the contained endosperm is increasing in size, first absorbing the contents of the cells of the integument and then crushing them by its growth. The embryo is also growing, absorbing the contents of the endosperm cells immediately surrounding it, and then crushing them. Sections through an ovary in which the embryo is half grown will show that whilst the cells of the endosperm around the embryo are being disorganized, active cell-division is still going on in the peripheral layers. The same is the case with the growing endosperm and the integument of the ovule. The cells of the outer limiting layer of the latter form few periclinal walls, but the whole constitutes a loose layer, whose cells divide into fours by anticlinal walls with subsequent separation of the daughter-cells.

The embryo is nursed at the expense of the endosperm, and the endosperm at the expense of the integument of the ovule, in the same manner as all such isolated growing bodies are nursed in the plant, *e.g.* the pollen in the anther, the pollen-tube in the style, and the young fern plant in the prothallium, by the production of a ferment or ferments which bring into solution the contents of the surrounding cells, and thus enable these contents to be absorbed.

Each cell of the innermost of the three mesophyll layers of the pericarp contains an octahedral crystal of calcium oxalate which is not stained by iodine, is not dissolved by acetic acid, but is dissolved without effervescence by nitric acid. The elongated cells of the outermost layer of the mericarp contain abundant starch-grains. This layer perishes in the ripe fruit, its outer walls being converted into mucilage.

53. Cut thin sections through a ripe mericarp, treat with iodine solution, and examine the contents of the cells of the endosperm and of the cotyledons of the embryo.

Neither contain starch. There is in each cell a ground substance of protoplasm containing numerous **Aleurone** grains, which are small rounded masses of a proteid derived from the cell protoplasm, and stored up in its vacuoles. These are stained a dark yellow by the iodine. If sections be cut dry and mounted in olive oil, a rounded body can be seen in the grain at one extremity. This is the **Globoid,** a body of a mineral nature. No proteid **Crystalloid,** such as are present in the aleurone grains of many other oily seeds, can be demonstrated here. The aleurone grains of Lamium are readily dissolved in a weak solution of common salt and in glycerine, but slowly and with difficulty in pure water. If sections, cut dry, be placed in Pfeffer's fluid—a solution of one part of mercuric chloride in fifty parts of absolute alcohol—for twelve hours or more, they can then be mounted unchanged in water or glycerine. Treat a section with osmic acid, and note by the dark coloration of the ground substance that it contains much oil. In the innermost cells of the endosperm the ground substance has disappeared, and the oil run together in drops. Dissolve it out with ether or chloroform. By fixing a ripe mericarp in a drop of melted sealing-wax by one of its outer angles on a cork, median longitudinal sections may be cut. Clear some in eau de Javelle, and examine the structure and position of the embryo.

The four mericarps constitute the **Fruit** of the plant. Each mericarp consists of the **Pericarp,** or outer wall, derived from the wall of the ovary, enclosing the **Seed.** In a great number of plants the pericarp ruptures on maturity, and liberates the seed. In Lamium this is not so. The seed is invested by the **Testa** derived from the integument of the ovule, and here represented by a layer of crushed cells only.

Germination.

54. Sow ripe mericarps of *Lamium album* in sandy soil. Keep moist, and examine day by day for the first signs of germination, the successive stages of which are as follow :—

The hypocotyl swells, elongates, and splits the pericarp, pushing out the radicle, which penetrates endosperm and testa. In whatever position the fruit lies, the radicle, which develops into the primary root, emerges at the micropylar end and grows downward into the soil. It attains a length of an inch or more, and develops numerous root-hairs shortly behind the apex, as well as the rudiments of secondary roots, before any other change is obvious. Then the hypocotyl elongates rapidly, and because the root on the one hand and the fruit on the other are fixed in the soil, it forms a curve convex upwards. A sudden upward growth of the lower part of the hypocotyl now draws the cotyledons out of the pericarp, and the hypocotyl straightens itself, lifting the cotyledons into the air. These are green when they emerge; their stalks now lengthen greatly, whilst the ventral surfaces of the cotyledons are still closely applied to each other as in the seed, but finally a quick growth of the ventral surfaces of the stalks separates them, they are spread out to the air and light, and function as the first foliage leaves of the plant. The cotyledons are round, being thus unlike the subsequent toothed foliage leaves. The transverse section of the hypocotyl is also round. During the stages of germination already described, the plumule remains undeveloped. It now grows in length, producing square internodes, nodes, and toothed foliage leaves similar to all subsequent ones.

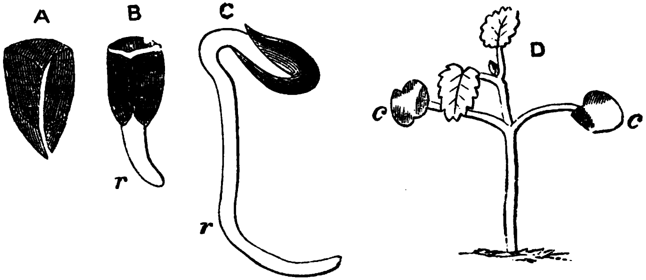

FIG. 134.—Germination of the seed of *Lamium album*. A, mericarp; B and C, stages of the germination; D, young plant; *r*, primary root; *c*, *c*, cotyledons.

Sections should be made through the germinating seed. These will show that the process of absorption of the endosperm by the embryo, which temporarily ceases on the ripening of the seed, is resumed on germination, and completed before the cotyledons leave the seed. This pause in the growth of the embryo is characteristic of the seed-plants, and seeds are prepared for the period of inactivity by the development of strong protective testas or pericarps. The normal resting period of the seed of *Lamium album* is short; if the conditions are favourable, development from oospore to the mature plant may be almost continuous and uninterrupted.

55. Remove a cotyledon from a seedling, clear in eau de Javelle, and mount whole in glycerine. Also cut longitudinal sections. Clear some, and examine others uncleared.

There are numerous stomata on both dorsal and ventral sides. Glandular hairs are plentiful on the sinuate epidermal cells. In the thickest part of the cotyledon there are about seven layers of mesophyll. Reckoning from the upper ventral surface, the first layer consists of long palisade cells with small intercellular spaces. Next are two or three layers of small cells, also with small intercellular spaces. In these layers lie the vascular bundles. Then two or three layers of larger rounded cells, with fairly large intercellular spaces. All contain chlorophyll. At the apex of the rounded cotyledon, opposite the stalk, is a single large water-gland, to which the anastomosing vascular bundles converge, and which thev enter. It has no water space, but is otherwise similar to those in the ordinary foliage leaf.

The development of the stomata can be easily followed from a surface view of the cotyledon. A wall perpendicular to the surface and convex inwards is first formed, and cuts off from an epidermal cell the **Initial Cell** of the stoma. Another wall, curved in the opposite direction, now appears in the initial cell, forming with the first wall a cell the shape of a truncated cone, perpendicular to the surface, and with its larger end outwards. This is the **Mother-cell** of the stoma, the remaining horseshoe-shaped portion of the initial cell being a **Subsidiary Cell.** The mother-cell is next bisected by a

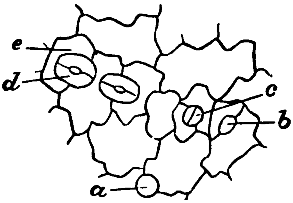

FIG. 135.—Stages in the development of stomata in the cotyledon of *Lamium album*. *a*, initial cell; *b*, mother-cell of stoma; *c*, *d*, guard-cells; *e*, subsidiary cell.

perpendicular wall, and a cleft appearing in this completes the formation of the stoma.

56. Prepare transverse sections from about the middle of the hypocotyl of a young seedling. Clear, and examine.

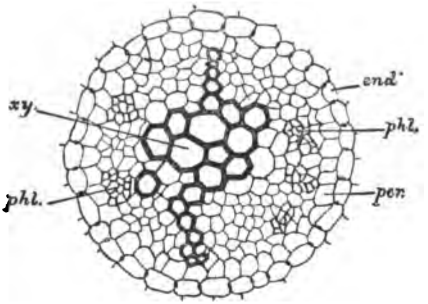

FIG. 136.—Transverse section through the middle of the hypocotyl of *Lamium album*. *end*, endodermis; *per*, pericycle; *phl*, phloëm; *xy*, xylem.

There are two bundles contained in an axial cylinder as in the root. A layer of endodermis, whose lateral walls when young show the characteristic dot, surrounds the cylinder. Within this is the pericycle. Xylem and phloëm are on alternate radii. Very early in the life of the seedling the two xylem masses meet in the centre of the hypocotyl; the phloëm spreads out laterally.

57. Prepare transverse sections of the stalk of a cotyledon, and examine.

There is a single vascular bundle in a flattened band running through the middle of the stalk. The middle of the band is occupied by the oldest wood vessels (spiral and annular), to the right and left are younger elements of the xylem, and outside these, still further to the right and left, are the phloëm strands. The arrangement, therefore, is different from that which obtains either in the stem or root.

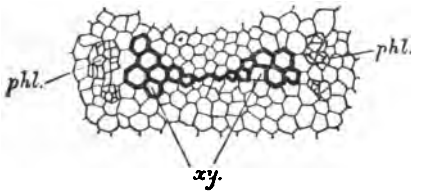

FIG. 137.—Transverse section through the stem of a cotyledon of *Lamium album*. The middle portion only is figured. *phl*, phloëm; *xy*, xylem.

58. Cut a series of transverse sections of the hypocotyl of a young seedling which has developed at least one pair of ordinary foliage leaves, commencing about one-third of an inch below the origin of the cotyledons, and extending into

the first internode. Clear, and mount them in glycerine in the order cut. Examine them in order, commencing with the upper sections, and make out the following points:—

The bundle of the leaf-trace of each cotyledon enters the hypocotyl obliquely and then bends abruptly downwards. The younger xylem moves towards the centre of the hypocotyl, the phloëm strands remain distinct for a time. These leaf-traces bound the axial cylinder of the hypocotyl on the sides of the cotyledons. Between them are the four bundles of the leaf-traces of the first pair of ordinary foliage leaves. A little lower these fuse with the leaf-traces of the cotyledons, two on each side, and at this level of the hypocotyl a transverse section shows two broad bands of phloëm, and within them the wood vessels loosely arranged nearly in a ring, the centre being occupied by parenchymatous pith. Lower still the vessels of the wood converge to form the flat band with the oldest crushed vessels at its poles, which is characteristic of the hypocotyl and primary root, and the phloëm bands are here also somewhat compressed laterally.

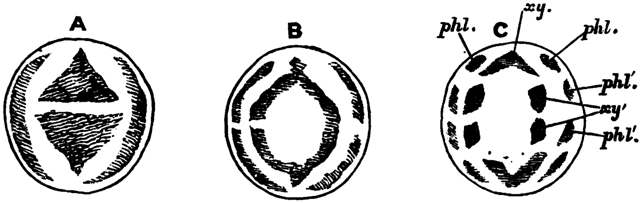

FIG. 138.—Transverse sections of the hypocotyl of *Lamium album*. A, above the middle of the hypocotyl; B, higher; C, just below the attachment of the petioles of the cotyledons. *phl*, *xy*, leaf-trace of one cotyledon; *phl′*, *xy′*, bundles from the first internode.

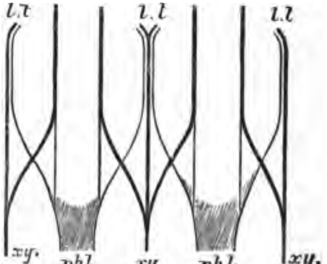

FIG. 139.—The bundle system of the hypocotyl of *Lamium album* projected on a vertical plane. *l.t*, leaf-trace bundles; *phl*, phloëm; *xy*, xylem.

Sections through the primary root show the same structure as in the hypocotyl. The origins of lateral roots may be well studied here, where the structure of the axile bundle cylinder is so simple.

Through the hypocotyl equivalent tissues in stem and root are in direct longitudinal connection and without torsion. The xylem and phloëm of the stem are continuous with the xylem and phloëm of the root, the endodermis of the stem with the endodermis of the root, the pericycle of the stem with the pericycle of the root, and cortex of stem with cortex of root. The abnormal structure of the bundle in the petiole of the cotyledon shows that the diarch axial cylinder of the hypocotyl really consists of two bundles, the phloëm of each of which is divided and placed to the right and left of its xylem, so that each phloëm plate contains elements belonging to each of the two bundles.

CHAPTER IX.

SPECIAL PHYSIOLOGY OF THE VASCULAR PLANTS.

YEAST, Protococcus, Chara, and other plants described live completely surrounded by a liquid which contains in solution everything necessary for the life of the plant, and there is no reason to doubt that almost all parts of these plants are equally capable of absorbing food-stuffs. The Fern and the Dead Nettle are in quite a different position with respect to absorption. Their roots are deep in the soil, their leaves high in the air, their internal structure is highly differentiated, and all parts are adapted to serve the life of the plant in the medium in which they are immersed.

It is a matter of common experience that such plants absorb certain substances from the soil and others from the air, and that their sub-aerial parts give off water into the air. By cultivating them so that their roots are immersed in Wolff's solution, it will be found that their food is the same in kind as that necessary for such a simple plant as protococcus. And it is obvious that since the root absorbs some nutrient substances and the green parts others, and since growth takes place mainly near the apices of stems and roots and where flowers, seeds, or spores are being produced, there must be currents of raw material as well as of manufactured (plastic) matter passing through all parts of the plant. It therefore remains to be described where and under what conditions mineral compounds, water and gases, are absorbed, and through what tissues they are conducted, the processes of constructive metabolism, the conduction of the products of constructive metabolism to places where they are used or stored, and the processes of destructive metabolism with the disposal of its products.

Absorption and Disposal of Water and Dissolved Substances.—If a young plant be placed under a glass bell-jar the inside of the glass becomes coated with water, especially if it be exposed to sunlight. If the sub-aerial portion of the plant be removed from the root and placed alone under a bell-glass the same thing happens, and in addition the plant becomes limp and flaccid in consequence of the loss of water. Not so, however, if the cut end of the stem be placed and kept in water. In that case there is no immediate withering. Water, therefore, escapes from the sub-aerial parts of plants; it rises from the roots, for if the stem be separated from the roots, and not put into connection with another supply of water, the plant withers; it must necessarily rise through the stem, and the chief amount of evaporation must be from the leaves. This loss of water is called **Transpiration**, and the water transpired is the excess of water absorbed by the roots over that required in the formation of new tissues. It is more active in sunlight than in darkness, in heat than in cold, in a dry atmosphere than in a moist one. It is not a mere physical process of evaporation, for the passage of water-vapour outwards is chiefly through the stomata and is controlled by their guard-cells, which, becoming turgid and swelling upward and outward, open the stoma in bright sunshine and close it by an opposite process in darkness. The air-cavities beneath the stomata are in connection with all the intercellular spaces of the plant, so that transpiration goes on from internal cells bordering the spaces. The cuticle, like all corky tissues, is impermeable to water, and transpiration therefore is mainly through the stomata. This loss of water from the leaves causes a suction from above which at a time of active transpiration reduces the pressure within the plant to less than that of the atmosphere.

If in a woody plant a ring of cortical tissue extending down to the wood be removed from the stem, the plant does not become flaccid, showing that the transpiration current is still kept up. Since nothing but the wood is left the current must rise through the wood.

Examination of the xylem of the vascular bundles of stem and root shows us, as we have already seen, that the tracheides are nearly full of air in the living plant. The spiral and annular unseptate vessels, however, which of all the lignified xylem elements penetrate nearest the growing apex, are there full of water; further from the apex they become crushed and disorganized. It is clear, then, from the structure of the tracheides which form the secondary wood of Lamium, and the nature of their contents, that the transpiration current travels to a large extent in their permeable lignified walls.

If the stem of an actively growing dead nettle be cut through near the soil a steady stream of water will exude from the woody portion of the stem, and this will continue for some time. This current cannot in this case be kept up by suction from above; there must be a pressure from below. This is the **Root-pressure.**

One reason for the great development of secondary wood in the stem of Lamium can now be seen; the increase in the volume of conducting tissue keeps pace with the increase in area of the transpiring surface—the leaves. In the fern no secondary wood is formed, but here transpiration is comparatively slight, and the plant possesses the power of forming new roots from and near the bases of the leaves themselves, so that the transpiration current in each leaf travels but a very short distance in the stem.

Transpiration may be so rapid in the dead nettle that the roots cannot absorb quickly enough to keep up the current. In such cases the pressure in the plant may become negative, and the leaves droop for want of water. On the other hand, when transpiration ceases or nearly so, as during the night, the root-pressure may still send currents of water up the stem, which passes into the lacunæ of the tracheides, and is forced out in drops from the pores of the water-glands at the teeth of the leaves. Such drops are not unfrequently to be seen on the leaves of *Lamium album* in early morning. The water glands therefore function as safety-valves.

We have studied three regions of the root—the root-tip covered with the root-cap; immediately behind this a region where the cells of the piliferous layer grow out into root-hairs; and behind this again the region where the piliferous layer has disappeared and the root is covered with the cuticularized exodermis. So long as the hairy portion of the root is in contact with water, the transpiration current is kept up. If the hairy portion be kept from water the plant will wither, whether the rest of the root be in water or not. The root-hairs then are the absorbing organs. They come into close contact with the soil, particles of which adhere to them with so much force that they cannot be removed without tearing the hairs.

The cell-sap of the root-hairs is acid. The soil-water contains substances brought into solution with the help of the carbon dioxide of the atmosphere and the acid cell-sap which diffuses out of the roots. The root-hairs then are in the position of the Protococcus cell. Cell-sap diffuses out of them into the soil, and water with dissolved substances diffuses into them. And since the hair contains osmotically attractive substances, especially organic acids, and its lining layer of protoplasm offers a greater resistance to exosmose than to endosmose, it becomes and remains turgid. Looking to the results, there is no doubt that the protoplasm lining that portion of the cell which borders on the cortical tissues, and of which the hair is an outgrowth, is in a different condition with respect to osmosis from the protoplasm of the hair. We have noted the same thing in the hyphæ of *Mucor.* The neighbouring cells of the cortex draw from the cells of the piliferous layer, and an inward moving current is thus set up towards the vessels of the wood where the pressure is least. Through these it rises and is disposed of in the manner described.

The transpiration current carries up with it, in solution, those mineral substances which are nutrient so long as they are being used. Non-nutrient dissolved minerals may also enter, but a state of equilibrium between those within and those outside the plant is soon reached and maintained, and no more is then absorbed.

The importance of the various elements of Wolff's solution to the life of the plant can be best studied by growing plants in the solution deprived of one or another of its constituents.

Nitrogenous compounds are essential to any growth, and the higher plants can as a rule make use only of nitrates as sources of nitrogen. The soil is constantly gaining nitrogenous compounds from the air and from decaying animal and vegetable substances in the form of salts of ammonia. These are oxidized first to nitrites, and then to nitrates, by the action of micrococci, which swarm in cultivated soils. It was long believed that plants cannot make use of nitrogen gas in contructive metabolism, and this is true with respect to the great majority, but there has lately been described a symbiotic relationship between some leguminous plants and the bacteria which cause and inhabit certain tubercles found on their roots. It has long been known that crops of leguminous plants may be taken off the land and leave the soil even richer in nitrogen compounds than before, and it is now thought that the bacteria enable their host to use free nitrogen, that is, to bring it into combination. The development of tubercles is directly related to the absence of assimilable nitrogen in the soil. Nitrates in the soil diminish the development of tubercles.

Phosphorus and sulphur are necessary for the formation of nucleoplasm and cytoplasm. Without iron, plants form no chlorophyll, and without potassium very little constructive metabolism goes on. Calcium and magnesium are essential, but their uses are not well known.

Absorption of Gases.—These, like solids, are only absorbed in solution. There is no doubt that carbon dioxide is absorbed in the water taken up by the root-hairs, but to an extremely slight extent compared with the amount taken up by the leaves and other green parts. Experiments have shown that much more carbon dioxide is absorbed by the upper surface of the leaf, where there are no stomata, than by the lower surface. Beneath the upper epidermis are the closely packed cells of the palisade parenchyma, and there the decomposition of carbon dioxide is most vigorous. The cuticle, impervious to water, is easily traversed by gases. The gas absorbed goes into solution in the cell-sap and passes from cell to cell by diffusion. The same must be said of oxygen, but with this difference: all parts of the plant, even the roots, absorb oxygen. In a water-logged soil healthy growth is impossible for most plants.

Metabolism.—A volume of oxygen, equal to that of carbon dioxide absorbed, is exhaled. So far as it is known the course of construction of proteid is of this kind. The carbon dioxide which is absorbed forms, in combination with water, a carbohydrate, an equal volume of oxygen being set free. This takes place in the chlorophyll corpuscles and under the influence of light. Once the carbohydrate is formed all the other steps of the process are independent of light. The nitrates absorbed by the roots are converted into ammonia compounds. The nitrates in a plant are found to decrease and the ammonia compounds to increase from below upwards. Ammonia combines with the carbohydrate formed in the chlorophyll corpuscles to form amides, and these combine further with some non-nitrogenous compound and sulphur to form proteids. From proteids protoplasm is constructed.

The oxidation and breaking down of protoplasm is incessant, and in the process carbon dioxide and water are formed and exhaled. Amides are also formed and heat set free. The amides are used again in the construction of protoplasm, and thus it comes about that in plants there is no excretion of nitrogenous waste matters as there is in animals. Starch is formed in chlorophyll corpuscles as in other amyloplasts by the breaking down of protoplasm, and so are fat, cellulose, and many others.

Proteids and the products of their decomposition are being continuously removed from the manufacturing leaves to the growing points and store places where they are being used. But proteids and starch are insoluble in any liquid the plant contains, and substances can only pass in solution from cell to cell. The removal of starch from the chlorophyll corpuscles is continuous. In the dark, when no carbon dioxide is being decomposed, the starch may be completely removed, only to appear again, however, with the light. There is in all green leaves a specific **Enzyme** called **Diastase,** which converts starch into grape-sugar. In this form it passes from the cells of the leaves into the parenchyma around the vascular bundles, and is conducted away through the cortex of stem and root to the places where carbohydrates are necessary to form protoplasm. Amides formed by decomposition of proteids are soluble and diffuse from cell to cell. The sieve-tubes of the phloëm contain proteids, and there is no obstacle during active growth to their passage through the tubes nearly to the apex of stem or root. The oldest portions of the vascular bundles of *Lamium album* are not wood vessels but sieve-tubes. Their companion cells appear to be glandular in nature. There is no doubt that the soft bast is a very important channel of conduction for organic substances.

The Correlation of Structure with Function.—We have seen that the root of a plant has a twofold office—to anchor the plant firmly in the soil, and to act as an absorbing organ. Because it is subject to tensile stresses the packing together of the strongest mechanical tissue of the root, viz. the tracheides of the wood, in the centre of the root is the best arrangement for the purpose. There too, they, being important conductors, are safest from injury. The root-cap, continually being worn off at its anterior extremity, and as continuously renewed from its formative layer, protects the delicate meristem at the apex. Without this it would be impossible for the root-apex to force its way through the soil. It is an advantage to the plant that the absorbing portion ot its root is readily renewable. A delicate, thin-walled tissue like the piliferous layer, is peculiarly open to injury from parasites, from the continued action of water, and from the roughness of the soil, and could not afford an adequate protection to the tissues of the cortex. It is soon replaced by the exodermis formed beneath it.

We have seen that the leaves must be well supplied with air and light in order that the plant may thrive. In *Aspidium* the short thick stem is mainly subterranean. The large leaves rise from it almost vertically to a height of two feet or more. But metabolism is not so active in the fern, which delights in shaded situations. In the dead nettle the stem is sub-ærial and erect, with its head of leaves equally distributed around it. We already know that, solid above where the tissues are soft and fleshy, it is hollow below, with its hardest elements—the tracheides of the wood—thrown into a hollow cylinder. It is a good mechanical principle that for a column which has to carry a weight, the same amount of material is better thrown into a hollow cylinder than formed into a solid rod. On the top of its hollow cylinder the head of leaves is carried. In the four angles of the stem are strands of collenchyma. By stripping them from a fresh stem it will be found that, whilst they possess little rigidity, they are very tough and elastic. A rigid stem of this size would be easily broken by the wind. The stem of *Lamium album* gives to every breath of wind and recovers its erect position immediately, the elasticity of the collenchymatous strands contributing greatly to this.

The leaf petiole makes an angle of about 60° with the stem, and carries the lamina at its distal end. Its ventral surface is hollowed out and the angles are occupied by strands of collenchyma. Its two largest bundles have well-developed wood, and as we have seen, the pericycle beneath all the bundles is largely increased in quantity and is collenchymatous. Here also, as in the calyx, the pericycle develops into a mechanical tissue. The hollow surface of the petiole and its strands of mechanical tissue make it specially suited to carry a weight at an angle. In the leaf itself, which is carried horizontally, the larger vascular bundles are accompanied by thick strands of mechanical tissue removed well below the surface of the leaf, and thus forming a most efficient support. The whole lamina also is tightly stretched by the vascular bundles, and is thus kept from flapping and tearing like the loose sail of a boat in the wind. It has already been pointed out how each leaf shades as little as possible the other leaves below it.

So long as the cells of the softer parts of the plant are turgid with water the tissues they constitute are as rigid as a bladder filled with liquid. Any cause which removes water, such as excessive transpiration, will render them limp and flaccid. A sharp blow or wrench will injure the cells and force water out of them into the intercellular spaces. This will cause a "set" for a time, until the injury is repaired.

The form and structure of the flower of *Lamium album* is a beautiful example of adaptation. Many investigators, and especially Darwin, have shown that it is not advantageous for most flowers that they should be fertilized by their own pollen, that is to say, **Self-fertilized.** By **Cross-fertilization** more seeds and seeds producing healthier progeny are secured. Self-fertilization is prevented in various ways. Some flowers are diœcious; in others the andrœcium and gynæcium mature at different times; and in others still the structure of the flower is such that self-fertilization is difficult or impossible. Cross-fertilization is effected in various ways, the most efficient instruments being insects who, visiting the flowers for pollen or honey, unconsciously carry pollen from one flower to another. This is the case with *Lamium album.* The honey is formed in the nectary and collects at the bottom of the corolla-tube below the ring of hairs. The projecting hood of the flower, well thatched with long hairs, protects that and the pollen from rain. Various insects, especially species of humble bees, visit the flower, alight on its lower lip, and thrust their bodies as far as possible into the corolla-tube in order to reach the honey with their trunks. In doing so, pollen-grains are shaken out of the anthers on their heads and backs, and are so carried to other flowers. Here the stigmatic surface at the end of the downward-projecting lobe of the style comes in contact with the pollen on the bee's back, and, retaining some on its sticky surface, cross-fertilization is accomplished. The ring of stiff upward-projecting hairs in the corolla-tube guards the honey from smaller animals which enter the tube and would take the honey without fertilizing the flower. The stronger bee can push its trunk between the hairs and so reach the sweet store beneath. The flower is also mechanically adapted to carry the weight of the bee. The adherence of the filaments of the stamens to the corolla-tube strengthens it exceedingly, the projection of the "throat" enables it to carry a weight vertically over it, and the strengthening of the calyx in the manner described is of the greatest service in this connection.

Flowers produced late in autumn sometimes do not open for want of warmth and sunshine. In this case it is not uncommon to find that the anthers dehisce and either shed their pollen on the stigma, or that even whilst in the loculus of the anther pollen-tubes are formed which reach the stigmas. In either case the flower is self-fertilized, and although this is not so advantageous to the plant as cross-fertilization, it is better than no fertilization.

It is not possible here to point out all the details of adaptability to function in *Lamium album.* It will form an interesting exercise for the student to seek out others for himself. In any case remember Darwin's injunction, "Give full play to your imagination, but rigidly check it by testing each notion experimentally."

CHAPTER X.

AMŒBA.

Amœbæ are found in standing water and on mud. They are universally distributed, but very aberrant in their occurrence. Many species are known, but several are no doubt but assumed amœboid forms of other animals and plants.

1. Mount water containing amœbæ, cover with a cover-glass, and examine.

They are difficult to distinguish at first by the unfamiliar eye on account of their being colourless and but slightly refractive. They appear as irregularly shaped splatches of a jelly-like substance, the shape constantly changing. It is easy to make out that there is an outer clear and tougher limiting layer, the **Ectosarc**, with granular more fluid contents, the **Endosarc.**

Measure. They will be found of various sizes, from 20μ up to 50μ.

2. Run in iodine solution.

The organism is killed; it shrinks into a globular form, and both ectosarc and endosarc are stained a yellowish brown, showing their protoplasmic nature. No blue colour is produced, showing that starch is absent.

3. Mount amœbæ in water, and place a small crystal of common salt at the edge of the cover-glass.

Nothing of the nature of plasmolysis occurs. The ectosarc is not a differentiated cell-membrane like that of the organisms already described.

4. Mount amœbæ in water, run in first a little weak acetic acid to kill them (1 per cent. solution), and next solution of magenta.

The dead cells stain with the magenta, and a rounded body in the endosarc deeper than the rest. This is the **Nucleus,** in which can sometimes be distinguished a **Nucleolus.** Not unfrequently more than one nucleus is present. The nuclear substance appears to be similar to that of plants.

5. Observe closely the endosarc of a living amœba. Some of the granular bodies contained in it are evidently foreign substances. Diatoms and cells of algæ are very frequently

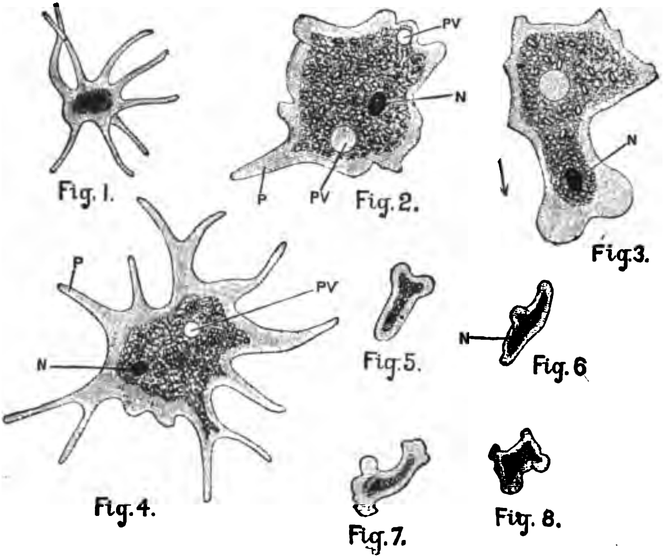

FIG. 140.—Amœbæ. 2 and 3 were drawn from the same specimen; in 4 the pseudopodia are branched; 5 to 8 were drawn from the same specimen at intervals of 20 seconds. N, nucleus; P, pseudopodia; PV, pulsating vacuole. (Marshall.)

present and are seen to lie in vacuoles surrounded by a watery cell-sap. In some of the larger amœbæ are seen **Pulsating Vacuoles** of the same character as those observed in Protococcus. The liquid which slowly fills them during their diastole is drawn from the general protoplasm of the endosarc, and is, during the systole, either returned to it or expelled from the cell. Here, also, the former appears to be the case, because at the systole the smallest particles of matter close to the cell appear to be unaffected by currents. More than one contractile vesicle may be present in large cells. They probably perform the function of a kidney, as uric acid has been detected in them.

6. Note the changes of form which the cell undergoes. Draw it at frequent intervals.

At a point where there was previously no projection a protrusion of the ectosarc occurs, the beginning of the movement being frequently extremely difficult to see on account of the portion first protruded being so little different in refractive power from the water in which the cell lives. The **Pseudopodium** enlarges and attains a considerable size, still composed wholly of ectosarc. Sometimes it is retracted, sometimes the endosarc flows into it, and the whole cell thus changes its position. In this way amœbæ crawl over the solid substratum. Pseudopodia may appear at many points of the surface at the same time, or at only one. In some amœbæ there is a region constantly free from pseudopodia. Branched pseudopodia may occur, and neighbouring ones may unite.

Its protoplasm is thus **Contractile.**

7. Note that the organism responds to stimuli of various kinds. Rough mechanical treatment causes pseudopodia to be retracted. Various chemical substances coming in contact with it have the same effect, as have also electric shocks. Contact with food-particles causes movement around the food, and when pseudopodia are protruded without apparent stimulus—**Automatic** movements—there is every reason to believe a stimulus is given from within. The movement following a stimulus is not necessarily proportional to the force employed, as it would be in the case of inanimate bodies, and this is the characteristic of **Irritability.**

Its protoplasm is thus **Irritable** and **Automatic.**

8. Watch the ingestion of solid food-particles.

Neighbouring pseudopodia either surround them, unite, and enclose them in the endosarc, or a region of the ectosarc comes in contact with them and the cell simply flows around them. In any case the particle enters where there was previously no opening, the hole made closing up again behind it, and the undigested portion passes out through an opening not pre-existent. Any portion of the ectosarc can receive food and allow excreta to pass through it, and any portion of the endosarc can form a digestive cavity for it.

Its protoplasm is thus **Absorptive.**

Some Protozoa are known which are, throughout the whole of their lifetime, almost completely enclosed in hard, structureless capsules or cysts. Oval shells, open at one end, through which the pseudopodia protrude which move the organism along, are characteristic of some species. The commoner amœbæ at times completely enclose themselves in spherical cysts of similar structureless formation, and in that condition can withstand unfavourable conditions of dryness, temperature, and so on, which would otherwise unfavourably affect them. The cyst is formed from the protoplasm.

Amœbæ may be frozen, but when the temperature is again raised they regain their power of movement. The optimum temperature at which movement is most vigorous is about 30° C. to 35° C. At from 40° C. to 45° C. they are killed by the coagulation of their protoplasm.

The whole surface of the ectosarc is an absorbing surface for substances in solution which pass into the cell by osmosis. But amœba cannot live in either Wolff's or Mayer's nutrient solutions, nor in any solution which does not contain suitable organic compounds of carbon and nitrogen. Being destitute of chlorophyll bodies, it cannot decompose carbon dioxide, but, like yeast and bacteria, it can live in a nutrient solution containing dissolved proteids (peptones). Unlike these latter, however, it takes solid proteids into its protoplasm, converts them into peptones, and assimilates them. The protoplasm of the living organisms which it swallows contains all the elements necessary for the formation of protoplasm in amœba, and since it has the power of dissolving this proteid food—that is, of digesting it—protoplasm answers all the requirements of a perfect and complete food for amœba, and supplies the organism as well with a store of potential energy. The course of constructive metabolism is thus shorter and simpler in amœba than in protococcus, the former commencing the course on material already highly organized.

Its protoplasm is thus **Constructively Metabolic.**

9. Cork up tightly in a bottle water containing amœbæ. They live but a very few days. Oxygen is necessary for the life of the organism, and when that contained in the water is all used up death ensues. The course of destructive metabolism is continuous, and does not differ in character from that described for other organisms. The effects are also the same: a supply of kinetic energy for movement and other manifestations of vital phenomena, and the keeping of the size of the cell within due bounds. The oxygen for that branch of destructive metabolism commonly spoken of as the respiratory process, and the carbon dioxide and other products of it, pass into and out of the cell by osmosis. The surface has thus, as in the other forms described, a respiratory significance. Protrusion of pseudopodia enlarges the respiratory area, oxygen is more largely absorbed, and the consequent production of kinetic energy increased. The cyst is a product of the destructive metabolism of the protoplasm, and so is the enzyme (unorganized ferment) which brings the proteids of its food into solution.

Its protoplasm is thus **Destructively Metabolic.**

Amœbæ multiply by fission. The nucleus first divides and then a constriction appears between the daughter-nuclei, which eventually cuts the cell in two. No other mode of multiplication is known.

Its protoplasm is thus **Reproductive.**

We have now seen that the protoplasm of amœba is **Contractile, Irritable, Automatic, Absorptive, Metabolic,** and **Reproductive.** The protoplasm of the zoospores of protococcus, and of many bacteria have also been seen to manifest these same properties. The protoplasm of yeast appears to be neither contractile nor irritable. How far this protoplasm is destitute of these properties it is impossible to say. The probability is that it possesses them, but that the mechanical restraint exercised by the cell-wall prevents their manifestation. It is instructive to note that in the encysted state amœba shows neither contractility nor irritability.

We may now make a statement of the income and expenditure of amœba in the following manner :—

INCOME.	EXPENDITURE.
Matter : Proteids and other constituents of protoplasm, oxygen, water.	*Matter :* Carbon dioxide, water, and other products of destructive metabolism. Excess of tissue-formation.
Energy : Heat. Potential energy of food.	*Energy :* Heat evolved. Work done in growth and movement. Potential energy of tissues formed.

BALANCE IN FAVOUR OF THE ORGANISM.

Matter : Excess of tissue-formation.
Energy : Potential energy of the same.

CHAPTER XI.

COLOURLESS BLOOD-CORPUSCLES.

1. KILL a frog by placing it under a glass with a piece of cotton wool saturated with chloroform or ether. Lay it on its back and slit up the integument of the abdomen with a pair of scissors. Lying in the middle line of the wall of the abdomen will be seen the **Anterior Abdominal Vein.** Pierce this and transfer a drop of blood to a slide. Cover with a cover-glass, and examine under a high power.

Lying amongst the crowd of pale **Yellowish-red Corpuscles** will be seen a very few, smaller than the coloured, which are destitute of colour. When first seen they are globular and very granular bodies, with no evident differentiation into ectosarc and endosarc and no pulsating vacuole (Fig. 183, p. 288).

2. Prepare a slide with a small drop of blood which does not extend to the edge of the cover-glass, and fasten the latter to the slide with melted wax applied all round with a brush, or with the end of a wax taper which has been warmed. This is to prevent evaporation. Keep warm and examine.

Notice that whilst the **Red Corpuscles** roll freely about in the fluid, the **Colourless Corpuscles** mainly attach themselves to the slide. At first the latter are spherical, but many of them soon begin to protrude hyaline pseudopodia. The change of form is, however, much slower than in amœba. By their amœboid movements the white corpuscles are able to pass through the walls of the blood-vessels.

3. Treat a drop of blood on the slide with weak acetic acid.

An irregularly shaped **Nucleus** will be brought into view lying near the centre of the cell.

4. Run in a drop of solution of magenta.

The nucleus is deeply stained.

5. Prick the tip of your finger with a needle, and mount a drop of blood.

Note the few colourless corpuscles in the crowd of red ones. If the slide be kept at the temperature of the body by means of a hot stage, their amœboid movements may be followed.

The colourless corpuscles of blood multiply by fission. They have been observed to engulf particles of colouring matter, such as carmine, which have been introduced under a cover-glass with them.

CHAPTER XII.

VORTICELLA (THE BELL ANIMALCULE).

VARIOUS species of Vorticella are to be found living in clear water, attached, solitary, or in colonies, to water-weeds or small animals, such as minute crustacea. When found in numbers the colony appears to the naked eye in bright sunlight like a multitude of small glistening beads.

1. Remove a piece of weed with the animals attached to it, mount in water, and examine first with a low, then with a high power.

The animal is campanulate, or bell-shaped, and is attached to its support by a long flexible stalk, which on the slightest irritation is thrown into a close spiral by which means the bell is drawn back to its support. Examine a fully expanded vorticella in detail, and note the following:—

(*a*) The edge of the bell is everted, and forms a projecting rim. This is the **Peristome.**

(*b*) The mouth of the bell is closed by a slightly projecting **Disc,** which, however, is not in contact with the peristome on one side, but leaves a groove between them.

(*c*) The groove passes a short distance towards the centre of the disc into the **Vestibule,** and the vestibule into the **Pharynx,** which, inclining underneath the centre of the disc, ends blindly there.

(*d*) A spiral wreath of **Cilia** runs round the margin of the disc and into the vestibule. The rhythmical contraction of the cilia causes a current of water to pass round the disc, to enter the vestibule and go down one side of it and up the other. These currents are visible by the movements of small bodies carried along in them. There are also cilia in the pharynx.

2. Tap the cover-glass slightly.

The disc is drawn in, the peristome closes over its edge and hides both cilia and vestibule. At the same time the stalk is thrown into a spiral, as already described, necessarily revolving the bell on its long axis in so doing. In a short time, if the irritation be not continued, the stalk slowly straightens itself. The bell opens out again, and the cilia once more get into motion.

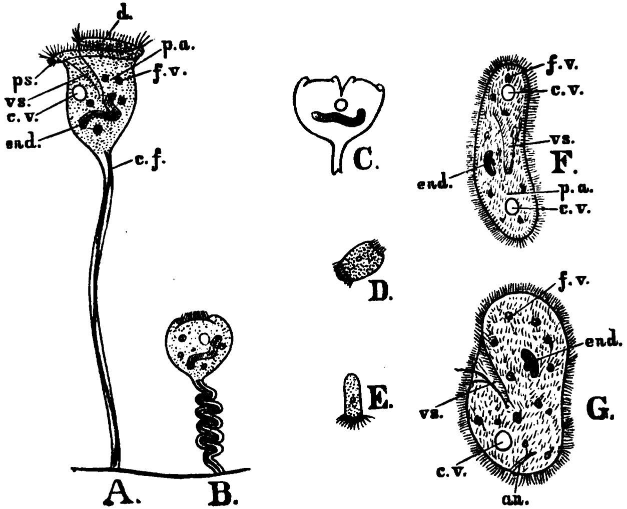

FIG. 141.—A, *Vorticella* extended. *c.f*, contractile fibre; *c.v*, contractile vacuole; *end*, nucleus; *f.v*, food vacuole; *p.a*, anus; *v.s*, vestibule; *p.s*, peristome; *d*, disc. B, *Vorticella* contracted; C, *Vorticella* in fission; D, free-swimming form after fission; E, Microzooid; F, Paramœcium; G, Nyctotherus. (Morgan.)

3. Examine the structure of the animal. Note—

(*a*) An extremely thin and delicate **Cuticle** investing it. This is thickest on the stalk.

(*b*) A layer beneath the cuticle, which shows a slight longitudinal striation and has been termed the **Myophan Layer.** The contractile muscle-fibre in the stalk is continuous with this layer. (*a*) and (*b*) together form the **Ectosarc.**

(*c*) The **Endosarc**, a granular and more fluid substance which fills the cell. The pharynx ends blindly in it.

In the endosarc note—

(*d*) **The Contractile Vacuole.** This is similar to that already described. It shows a slow diastole, during which the vacuole is filling with water, and a rapid systole, during which it becomes emptied and its walls collapse. It probably expels from the cell water charged with nitrogenous waste. It has been observed to extract from the endosarc soluble colouring matter taken in with the food.

(*e*) Various **Food Vacuoles.** These consist of particles of food which have been ingested with a drop of water. They exhibit a slow movement of rotation (**Cyclosis**) through the endosarc.

(*f*) The **Nucleus**, which is readily visible after staining with iodine or fuchsine. It is large and band-shaped, and is placed near the pharynx.

4. Watch for the expulsion of undigested substances. They are thrust through the ectosarc into the vestibule by an opening which is visible only at the moment of the ejection of fæcal matter. This opening is the **Anus.**

5. As a protection against adverse influences, such as want of food or the drying up of water, **Encystment** frequently occurs, and for a while the animal hibernates. The disc is drawn in, the peristome inverted, the individual separates from its stalk, rounds itself off, and invests itself with a structureless envelope, or **Cyst.** On the return of more favourable conditions it resumes its ordinary mode of life.

6. **Reproduction** is by means of **Fission.** A cleft forms on the disc and gradually extends to the point of attachment of the stalk, passing through vestibule and nucleus. The half of the bell attached to the stalk quickly recovers the form of the mature animal, the other half develops a circle of cilia at its aboral end, detaches itself from the parent stalk, and after swimming free for a time comes to rest, withdraws its aboral cilia, attaches itself by its base to some fixed object, and develops a gradually lengthening stalk.

There is occasionally a process of **Conjugation** of unlike gametes. The larger—which, if we look upon this as a sexual process, may perhaps be termed the female—is the ordinary stalked form; the smaller is a free-swimming form, and may be termed the male. The latter (**Microgonidia**, or **Microzooids**) are formed by the repeated vertical fission of an ordinary individual producing eight small free-swimming forms which move through the water by the action of an aboral circlet of cilia. Each attaches itself to the base of a female, and complete fusion follows. In the process the nucleus of each gamete divides and subdivides, but all daughter-nuclei except one in each gamete abort. The pronuclei which remain move towards each other, meet, and fuse to form the single nucleus of the new individual. This may be looked upon as a process of **Rejuvenescence**, as fission follows after the conjugation with great frequency.

Encystment sometimes follows conjugation, and it is said that during this encystment the nucleus breaks up into a number of free-moving **Spores**, each of which escapes by rupture of the cyst and develops into an ordinary Vorticella.

7. **Nutrition.** Although it is not improbable that Vorticella may absorb nutrient substances in solution by its whole surface, yet its structure shows that it is adapted for feeding on solid food. The wreath of cilia on the disc causes a current in the water, which draws towards it small bodies in suspension. These are swept into the vestibule, and thence into the pharynx, where they accumulate at its blind end. The pellet is then forced through the ectosarc into the endosarc, together with a small drop of water in which it lies. In the course of the rotation of the food vacuole through the endosarc, the digestible portion is brought into solution, and the remainder is ejected through the anus in the manner already described. The ingestion and subsequent migration of solid substances can be studied by placing finely divided carmine in the water with Vorticellæ.

The absorption of oxygen and elimination of carbon dioxide takes place over the whole surface of the animal. Gases in solution are also of course taken in with the water accompanying food-particles. The excretion of nitrogenous and other waste is effected most probably by the contractile vacuole.

8. **Motion.** Cyclosis in the endosarc has already been noticed. Cilia are delicate protoplasmic processes whose rhythmic motion of quick contraction of one side and somewhat slower straightening is continuous. Whilst the animal is fixed in position, their co-ordinated movements have for object the procuring and ingestion of food; when freed from its stalk both adoral and aboral cilia cause locomotion. The contraction of the muscular band in the stalk which throws the latter into a close spiral is intermittent, and follows external irritation, whilst the motion of cilia is automatic. The straightening of the stalk is apparently effected by the elasticity of its cortical layer. No specialized nervous matter is known to be present; the whole organism is a neuromuscular cell.

Morphologically, Vorticella is a single cell, but one whose differentiation has reached a higher degree of complexity than that of any tissue-cell of the higher animals or plants. Unicellular animals like Amœba, Paramæcium, and Vorticella belong to the great group of **Protozoa**, the lowest grade of the animal kingdom. Not all the Protozoa are unicellular, but the individual cells of the multicellular forms are all alike in structure and function, and may be separated from each other and live independent lives. The connection of the individuals in such a "colony" is not an organic connection, but is the mere cohesion of the offspring of unicellular forms which have not separated from each other.

CHAPTER XIII.

PARAMÆCIUM AURELIA (*THE SLIPPER ANIMALCULE*).

THIS, like Vorticella, is a unicellular animal, very commonly found in pond water and in vegetable infusions. Its trivial name sufficiently describes its form.

1. Mount a drop of water containing Paramæcia with some threads of cotton wool to restrict their movements, and examine first with a low, then with a high power.

Note its shape, blunt at one end, sharper at the other, and flattened laterally. Its backward and forward movements are effected by means of cilia, which cover the whole of the body. On the oral side is a groove leading into the tubular **Pharynx, Oral Groove** and pharynx being supplied with cilia.

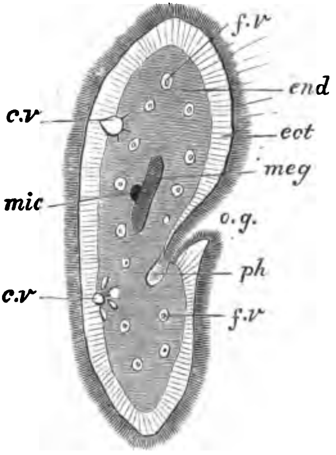

FIG. 142.—*Paramæcium aurelia.* *o.g*, oral groove; *ph*, pharynx; *ect*, ectosarc; *end*, endosarc; *c.v*, contractile vacuoles; *f.v*, food vacuoles; *meg*, meganucleus; *mic*, micronucleus.

2. Note the very definite **Ectosarc.**

(*a*) A delicate **Cuticle,** through which project the cilia, covers the animal, extending into the pharynx.

(*b*) In the vertically-striate layer beneath the cuticle are a number of oval sacs containing spirally coiled threads. These are the **Trichocysts,** and from them the stiff threads are shot out when the animal is irritated. A drop of acetic acid drawn under the cover-glass will bring about this result.

(*c*) Two **Contractile Vacuoles,** also in the cortical layer, immediately beneath the cuticle. They are on the aboral side, and at some distance from each other. Leading from the vacuoles like the rays of a star are canals passing into the endosarc. Canals and vacuole contract alternately. At the completion of the systole of the vacuole the stellate canals are full, during its diastole they empty.

(*d*) An **Anus** appears just below the mouth at the moment of ejection of fæcal matter.

3. Note in the more fluid **Endosarc**—

(*a*) **Food Vacuoles** exhibiting **Cyclosis,** as in Vorticella.

(*b*) Two **Nuclei** lying behind the pharynx, a very large **Meganucleus** and a much smaller **Micronucleus** beside it. The larger is sometimes spoken of as the **Nucleus,** the other as the **Paranucleus.**

4. The **Nutrition, Respiration,** and **Excretion** of Paramæcium are in all respects similar to these processes in Vorticella.

5. **Reproduction.** Paramæcium reproduces itself by transverse fission. A cleft appears opposite the mouth and extends across the animal, mega- and micro-nuclei being both divided so that each product of the fission has a portion of each nucleus.

There is also a process of **Conjugation** which differs considerably from the conjugation of Vorticella. The conjugating gametes unite by their ventral surfaces, and may remain in conjugation for several days. During this time the meganucleus in each animal becomes fragmented by mere constriction (a process which we have already called direct nuclear division), and its fragments sometimes completely disappear. Each micronucleus divides twice (by indirect nuclear division), thus producing four daughter-nuclei. Three of these (rejection nuclei) abort, the other divides again into two pro-nuclei, one of which is stationary, the other migratory. Each migratory pronucleus passes from one conjugating animal to the other, and there fuses with the stationary pronucleus, but there is no transference of cytoplasm, conjugation being wholly a nuclear process. The animals then separate. The conjugation-nucleus in each divides twice, producing two mega- and two micro-nuclei, one of each passing into the two individuals formed by the fission which immediately follows. This is also therefore a process of **Rejuvenescence.**

There is reason to believe that the two nuclei of Paramæcium, as well as of other unicellular animals (*Protozoa*) of the same grade (*Ciliata*) are together the equivalent of the single nucleus of other cells, and that in the meganucleus reside the physiological properties of a nucleus, whilst the hereditary properties are contained in the micronucleus. It will not be uninstructive in this connection for the student to recur to what has been said before about the fragmentation of the nucleus in those cells of the *Characeæ* which have for ever lost the power of division.

CHAPTER XIV.

HYDRA VIRIDIS.

THERE are three species of hydra found inhabiting still fresh waters in this country, the commonest being the green *Hydra viridis.* They are all small, although not microscopic, seldom exceeding when fully extended a length of half an inch, and when retracted looking like small rounded particles of jelly. They will be found attached to water-weeds by a foot which acts like a sucker, or hanging free with the foot at the surface of the water and tentacles spread out below. They thrive well in a small window aquarium with water-weeds, and show their liking for light by crowding together on that side of the aquarium which is nearest the window.

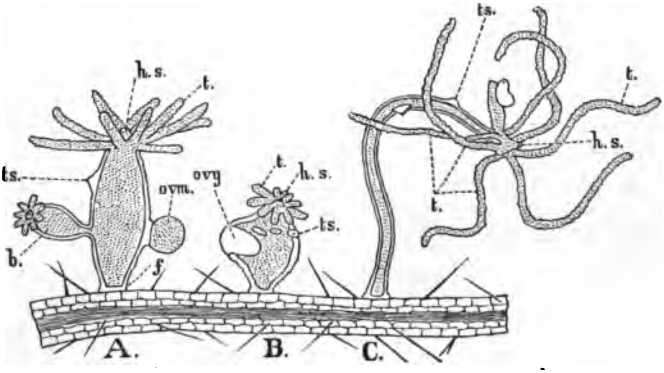

FIG. 143.—*Hydra viridis.* A, hydra half retracted; B, hydra fully retracted; C, hydra fully extended; *f,* foot; *ovm,* ovum; *b,* bud; *ts,* testis; *h.s,* hypostome; *t,* tentacle; *ovy,* ovary. (Morgan.)

1. Transfer some Hydras to a shallow vessel, and keep

them under observation for a time, using a hand lens when necessary.

The animal consists of a long cylindrical body, which when fully extended is of about equal diameter throughout. At one end is the **Foot,** by which it attaches itself to solid objects or to the surface film of the water. It can also shift its place slowly by means of the foot. At the other end is the **Mouth,** not visible with the naked eye, around which are arranged from six to eight **Tentacles.** These are freely movable, constantly bending, straightening, contracting, and expanding. Touch the tentacles and they immediately contract. Watch a small animal come in contact with them, and they bend over on it, carrying it to the mouth. The body also is commonly in motion, slowly bending first to this side, then to that. Frequently similar but smaller animals are attached to the body of a Hydra. These are **Buds,** which are outgrowths of the body-wall of the parent. Smaller projections may also be found on the outside of the body-wall. These are the **Sexual organs.** Frequently the animal changes its place by a looping motion like that of a caterpillar, taking hold alternately by means of foot and tentacles. The whole animal is a hollow bag, the cavity extending throughout the whole of its body and into the tentacles.

2. Mount one in an excavated cell, or on an ordinary glass slip with a piece of weed to prevent pressure, and examine with a low power.

Note the points already alluded to. The mouth is situated at the apex of a conical **Peristome,** at the base of which is inserted the ring of tentacles. When the animal turns its peristome upwards the mouth can be easily seen. It is the only orifice leading into the body. The tentacles especially, and the body-wall to a less extent, present a knotted appearance.

3. Examine with a higher power an extended tentacle.

Focus the margin, and note that on the outside is a colourless layer. This is the **Ectoderm,** which is sharply separated from the internal green layer, the **Endoderm,** by a thin structureless and colourless layer, the **Supporting Lamella.**

(*a*) The **Ectoderm** consists of—

i. A layer of large cells, which when the tentacle is extended are seen to be very long, narrow, shallow, and flat on the surface; when the tentacle is contracted they are short, wider, deeper, and rounded on the surface. They are the **Neuro-muscular** cells of the ectoderm.

ii. In and between these large cells are smaller bodies collected together in groups, and some of them projecting from the extended tentacle so as to give it a knotted appearance. They are the **Interstitial** cells, small naked nucleated masses of protoplasm, and certain products of their differentiation. The latter are of three kinds—

a. **Nematocysts,** or **Thread-cells.** These are ovate sacs with truncated outer ends, and filled with liquid. Some will be seen with everted hollow whip-like threads, barbed at the base, the thread being a continuation of the wall of the sac; in others the threads are coiled up in the sac. They are weapons for capturing, and perhaps paralyzing small animals which form the food of the Hydra. In eversion the thread

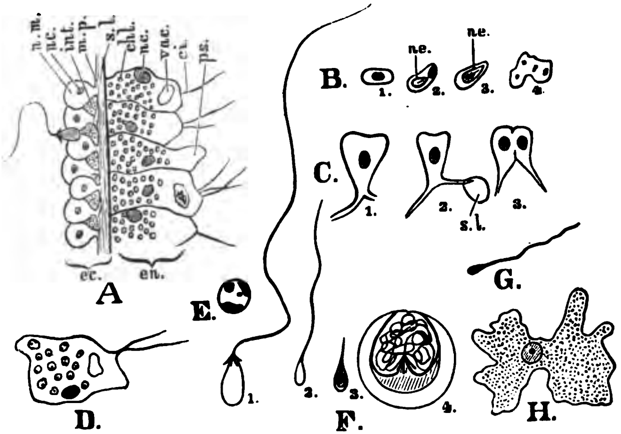

FIG. 144.—*Hydra.* A, longitudinal section through the body-wall; *ec,* ectoderm; *en,* endoderm; *n.m,* neuro-muscular cells; *nc,* nucleus; *int,* interstitial cells; *m.p,* muscle process; *s.l,* supporting lamella; *chl,* chlorophyll corpuscles; *nc,* nucleus; *vac,* vacuole; *ci,* cilium; *ps,* pseudopodium. B, interstitial cells; *ne,* nematocysts; C, neuro-muscular cells; D, endoderm cell; E, chlorophyll corpuscle; F, nematocysts; G, spermatozoon; H, ovum. (Morgan.)

is darted swiftly into the body of the prey, being turned completely inside out in so doing. In fortunate cases the observer may find small animals fixed to the tentacles by nematocysts. After capture the prey is carried to the mouth and swallowed.

β. Thread-cells, as long as the other actively offensive nematocysts, but narrower, and with shorter and thicker unbarbed threads. They, like the thread-cells described above, are surface cells. These are in great numbers on the peristome about the mouth.

γ. Much smaller thread-cells, of the same shape as the active cells, but with fixed short threads. Whilst the actively offensive nematocysts project from the surface on maturity, these always lie deep among the bases of the neuro-muscular cells. They are not improbably tactile bodies.

(*b*) The **Endoderm** consists of a single layer of large cells whose bases are everywhere in contact with each other on the supporting lamella, but whose free internal borders are amœboid, and capable of extending, so as to almost close the cavity of the tentacle, or of contracting, so as to form a layer little thicker than that of the ectoderm. The undulating border of the endodermal layer of cells can be seen in the tentacle with here and there a moving **Flagellum.** Chlorophyll corpuscles are present in large numbers.

The structure of the body is similar to that of the tentacles with, however, fewer thread-cells.

4. Tease up a fresh Hydra in water, add a trace of methyl blue, and examine with a high power. Several such preparations must be made before all the following points are made clear.

(*a*) The neuro-muscular cells are rounded on the wide outer ends, but their narrower inner ends are produced into long processes which lie along the supporting lamella. Each contains a large nucleus, which stains well. In the intercellular spaces at their bases lie the interstitial cells.

(*b*) Rounded interstitial cells, set free by the separation of the large neuro-muscular cells, float freely in the water. Their small nuclei stain deeply.

(*c*) The large offensive thread-cells. Their development can easily be traced in the globular interstitial cells (**Cnidoblasts**) in which they are found. When mature the much-reduced cnidoblast still envelops the sac, and in the larger variety projects beyond its apex on one side as a small-pointed process called the **Cnidocil.** The other forms of thread-cells will also be isolated.

(*d*) Sometimes small, isolated, deeply stained stellate nerve-cells.

(*e*) Portions of the supporting lamella should be looked for.

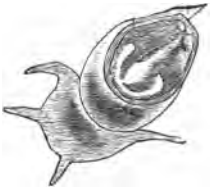

FIG. 145.—Undischarged nematocyst of *Hydra*.

(*f*) The **Endodermal** cells are very large and profusely vacuolated. In the cells, or set free from torn cells, will be found great numbers of chlorophyll corpuscles. In the cell they lie on that side adjacent to the supporting lamella. The green colouring matter is not equally diffused throughout the corpuscle, but occupies certain definite spaces in it, chiefly in its periphery. Small vesicles which have escaped from torn endodermal cells may be found with chlorophyll corpuscles in them, and in every cell are one or more small vesicles containing small dark-brown granules, which may be disorganized chlorophyll bodies. Small particles of food, diatoms, and portions of the chitinous cases of small animals are also frequently to be found in the cells.

5. Inundate a Hydra with very weak (·5 per cent.) acetic acid. Tease this up, and examine with a high power.

The nematocysts will be found with everted threads. The interstitial cells are separated, and especially well seen in such preparations.

6. Kill a fully extended Hydra by suddenly inundating it with 1 per cent. osmic acid. Wash in water, place in alcohol for a few hours to dissolve out the chlorophyll, wash again in water, and stain in dilute ammonio-acetic carmine (Hamann's). Mount in water without pressure, and examine.

Select the most extended portion of the body, usually just above the foot, focus the surface first, and then take successive optical sections through to the endoderm. Note the well-stained stellate nerve-cells which are very rarely met with in close connection with nematocysts, the long processes of the neuro-muscular cells, the interstitial cells in groups, the longitudinal striation of the supporting lamella, the outlines of the bases of the endodermal cells forming a lattice-work and the contents of these latter cells.

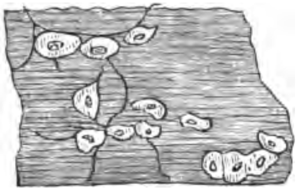

FIG. 146.—Supporting lamella of *Hydra*, with interstitial cells lying on it.

7. Tease up a Hydra, treated as in 6, in water or dilute glycerine, and examine with a high power.

Note the wavy fibrillæ of the supporting lamella. They lie parallel to one another in the direction of the animal's length. In some places they may have been separated from one another by the teasing.

Nutrition.—The animals caught by the tentacles are carried to the mouth. This is very extensible, and on the approach of the captured prey it opens widely, showing its five lobes or lips. These seize the prey and force it into the cavity of the body, which is the digestive cavity. A Hydra is capable of swallowing an animal of much larger diameter than itself. In the digestive cavity the food is broken up, the softer parts are dissolved and absorbed in solution by the endoderm cells, the harder parts are forced into these cells by their amœboid activity, and are there subjected to intra-cellular digestion. The flagella

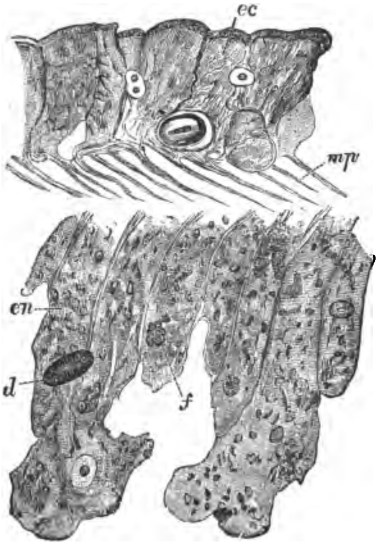

FIG. 147.—Longitudinal section through a *Hydra* killed during digestion. *ec*, ectoderm; *en*, endoderm; *mp*, neuro-muscular processes; *d*, *f*, food-particles. (Parker.)

of the endodermal cells keep the fluid of the digestive cavity constantly in motion; the current can be easily observed in the tentacle of the living animal. The cells outside the supporting lamella are nourished by diffusion from the endodermal cells. Fæcal matter is ejected through the mouth.

The part played by the chlorophyll bodies in nutrition, and their relationship to the animal, is not clearly understood. Starch is not found in them, although they liberate oxygen from carbon dioxide. There is not much reason to doubt that they are concerned in the formation of protoplasm here as in the green plant.

There is not known to be any definite **Nervous System** in Hydra. Stellate cells, which have been described as similar to the nerve-cells of the higher animals, occur among and between the groups of interstitial cells, but the complex movements which the animal is capable of are possibly combined, as they are certainly produced by the processes of the neuro-muscular cells.

Reproduction is both **Asexual** and **Sexual.**

Asexual Reproduction is by means of buds produced from the body of the well-fed parent. The bud is a product of both ectoderm and endoderm. It appears first as a lateral protuberance of the body-wall, increases rapidly in length, tentacles are developed by outgrowths near the extremity, and a mouth is formed at the apex of the peristome. The bud leads an independent life whilst it is still in organic connection with its parent, and it can even become the parent of other buds before separation takes place.

A Hydra may be cut in two pieces and each piece will develop into a perfect animal. Fission, however, is not known to take place naturally.

Sexual Reproduction is of an advanced type. The animal is monœcious and self-fertilizing. The **Ovary** is a large spherical projection of the body-wall, usually not far above the foot, and formed by local growth of interstitial cells. One of these becomes much larger than the others, being nourished at their expense, and is amœboid. This is the **Ovum,** which has a well-defined nucleus (germinal vesicle) and nucleolus (germinal spot). The nutrient cells form the ovarian wall, which ruptures at the apex when the ovum is ripe so as to expose its apex, and in this position it is fertilized by spermatozoa from the testis of the same individual. The ovum of *Hydra viridis* contains chlorophyll corpuscles.

The **Testis** is a much smaller swelling of the body-wall near the tentacles. Whilst in *H. viridis* more than one ovary is rarely found, there are usually several testes. These are also formed by active subdivision of interstitial cells, the wall of the testis being formed by the large ectoderm cells. The spermatozoa are long thread-like bodies with highly refractive heads formed from the nucleus of the mother-cell, and long swimming tails formed from the cytoplasm of the mother-cell. They escape when ripe by rupture of the wall of the testis. To set them free for examination it is only necessary to tear off the capsule of a large testis with a fine needle, or apply pressure to the cover-glass.

After fertilization the ovum by repeated divisions forms a spherical mass of cells. This becomes coated by a thick, rough chitinous coat secreted by the outer cells of the mass, and in this state it falls into the mud and develops into a young hydra.

Hydra is one of the simplest representatives of the **Metazoa** or **Enterozoa** which differ from the Protozoa in the differentiation of structure and function of its constituent cells. The Metazoa includes all animals above the grade of Protozoa, and all are characterized by ectodermic and endodermic layers of cells and the possession of a digestive cavity (**Enteron**) communicating with the exterior.

CHAPTER XV.

THE POND, OR SWAN MUSSEL (ANODONTA CYGNEA).

THE common Pond, or Swan Mussel is an inhabitant of fresh-water ponds, canals, or slowly running rivers. It imbeds itself in the mud or sand at the bottom by means of a muscular retractile foot and always in such a position that the axis of the animal forms an acute angle with the surface. In shallow, clear, unshaded water with a soft bottom it sinks in the mud until nothing is left exposed but the extreme posterior dorsal end of its shell; in deep or shaded water a much larger portion is left uncovered. By means of its foot it can move slowly about from place to place.

The mussel feeds on minute organisms, both plants and animals, suspended in the water. It is incapable of rapid movements to seize its prey, and it is not furnished with any organs of prehension, but by means of the vibratile cilia, with which large portions of its body are covered, it produces a current in the water which brings to its mouth the minute organisms on which it feeds, supplies it with aërated water for respiration, and carries away from it fæcal matter.

Procure living mussels, place some in water with a layer of clean sand at the bottom, and put others into a quarter per cent. solution of chromic acid for a couple of days and then into alcohol. The mussel may be killed for dissection with its foot protruded by placing it in cold water and gradually raising the temperature to about 150° F.

1. Watch the mussels in water. The **Exoskeleton** is bilaterally symmetrical, and consists of two hard **Valves** hinged along the dorsal line, and capable of being separated

along the ventral line. Each valve shows excentric markings curving around a boss on the dorsal side. This boss is the

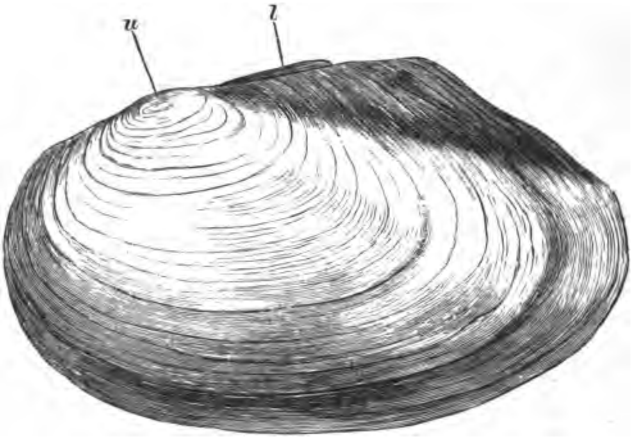

FIG. 148.—Outside of valve of *Anodonta cygnea*. *u*, umbo; *l*, ligament.

Umbo, and is the oldest part of the shell; the curved lines are lines of growth. It will be readily seen from the lines of growth, that growth proceeds more rapidly towards one extremity of the valve than towards the other. The extremity of more rapid growth is the posterior extremity; the opposite is the anterior.

Out of the water the valves of the living mussel are closely approximated on the ventral side; in a dead mussel they gape widely. The valves of the living mussel are kept closed by the contraction of **Adductor Muscles,** which pass straight across from one to the other; when they are not contracted the valves open by mechanical means. Above the hinge-line, just posterior to the umbones, is a raised dark-coloured ridge. This is the **Elastic Ligament,** which, projecting to the dorsal side of the hinge, converts the valves into levers moving on the hinge as fulcra. When the valves are closed the ligament is stretched; on relaxation of the adductor muscles the elasticity of the ligament opens the valves. Cut longitudinally through the elastic ligament of a living mussel, and the valves separate no more. The injured ligament is never repaired.

Watching a living mussel in the water, it will be seen to open its valves, protrude its foot—a reddish-yellow mass—which, bending over to the sand and burrowing in it, turns the dorsal side of the animal uppermost. Then at its posterior extremity currents appear in the water. Rub up finely-divided carmine in water, take a little in a dipping-tube and release a few drops close to the posterior extremity of the mussel. It will be drawn between the valves through an opening fringed with short **Tentacles,** and a moment after a portion of it will be expelled from another orifice close above the first. This current is constant so long as the valves are open in water. The ventrally placed orifice fringed with tentacles is the **Inhalent Siphon.** The tentacles form a strainer which prevents large particles from entering. They are very sensitive, and when a larger body than ordinary comes in contact with them the valves are quickly closed, expelling a large quantity of water and the offending particle with it. The orifice above the inhalent siphon is the **Exhalent Siphon.** Its borders are thin and mobile, and not unfrequently they alone close the siphon, causing the exhalent current to emerge through a

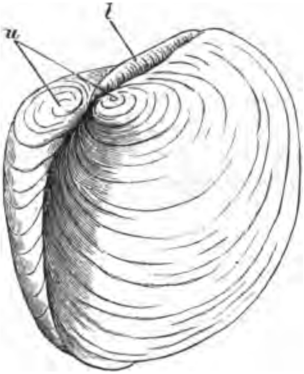

FIG. 149.—*Anodonta cygnea.* *u*, umbones; *l*, ligament.

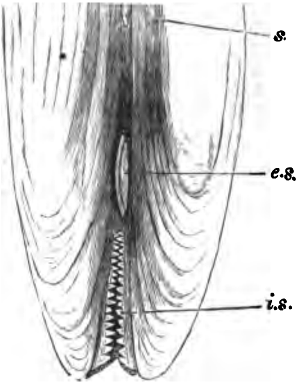

FIG. 150.—*Anodonta cygnea;* posterior extremity. *i.s*, inhalent siphon; *e.s*, exhalent siphon; *s*, supplementary opening.

smaller orifice, or even more than one, dorsal and anterior to the exhalent siphon.

2. Take a living mussel, prise open the valves to a slight extent, and, looking inside, note that the inner surface of each valve is closely covered by a membrane whose free surface extends nearly to the edge of the valve. This is the **Mantle,** and the space between the two mantle-lobes is the **Branchial Chamber.** In the branchial chamber are suspended four **Gill-plates,** two on each side of the median foot. The inhalent siphon leads into this chamber, the exhalent siphon leads into a **Supra-branchial Chamber** overlying the branchial chamber, and communicating with it by two large slits further forward, and by numerous pores in the gill-plates.

3. Lift the edge of one mantle-lobe, and note that it is attached to the valve some little distance from its edge along the whole length of the valve. This is the **Pallial Line.** Near the posterior extremity the attachment is firmer. This is where the **Posterior Adductor Muscle** is inserted on the valve. Cut through this muscle close to the shell with a sharp knife, detach the mantle-lobe along the pallial line, and cut through the **Anterior Adductor** and other muscles close to

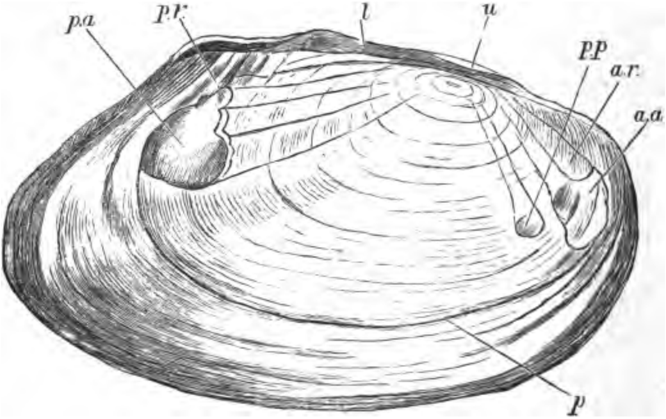

FIG. 151.—Inside of valve of *Anodonta cygnea*. *p*, pallial line; *p.a*, posterior adductor; *p.r*, posterior retractor; *a.a*, anterior adductor; *a.r*, anterior retractor, and *pp*, protractor pedis impressions; *u*, umbo; *l*, ligament.

it in the same way as the posterior. The valve may now be separated from its fellow along the hinge line, leaving the animal lying in the other valve. Place it in water in this position.

4. Examine the detached valve. The inner surface except along the edge has a pearly lustre. The pallial line and the lines of growth are clearly marked. Near the extremities are the impressions of the attachment of the muscles. Compare these impressions with the positions of the muscles themselves in the animal lying in the other valve, and notice—

(*a*) The **Anterior Adductor** impression. This is a large impression lying nearly close to the anterior extremity of the valve. The contraction of the muscle closes the valves.

(*b*) The **Anterior Retractor** impression is small, and lies immediately behind that of the anterior adductor and continuous with it. The muscle withdraws the foot after protrusion.

(*c*) The **Protractor Pedis** impression is small, and lies a short distance below and behind that of the anterior adductor.

(*d*) The **Posterior Adductor** impression is large. The muscle closes the valves by its contraction.

(*e*) The **Posterior Retractor** impression is small and continuous with the dorsal limit of the posterior adductor impression.

The muscular impressions are the bases of faintly outlined triangular spaces whose apices are at the umbo. They mark the lines along which the muscles shifted as the animal grew.

5. Notch the valve with a file from the umbo to the ventral surface, break it across, and examine the edge with a hand lens. For the details of structure thin sections must be examined. Note that it consists of the following three layers :—

(*a*) The **Periostracum** is the thin, horny, brown outer layer. This is reflected at the ventral margin over the inner edge of the valve.

(*b*) The **Prismatic Layer** occupies the middle half of the thickness of the shell. It is composed of polygonal prisms of calcium carbonate placed closely side by side. This and the periostracum are secreted by the free edge of the mantle.

(*c*) The **Pearly Inner Layer** is composed of alternate laminæ of calcified and uncalcified matter. It is secreted by the whole inner surface of the mantle.

6. Place a piece of the valve in dilute hydrochloric acid. The calcium carbonate is dissolved with evolution of gas, leaving the soft animal matter of the shell.

7. Burn a valve in a clear fire, remove, and examine when cold. The animal matter has been consumed, and in consequence the inner layer easily separates completely from the middle layer along the pallial line and breaks up into thin flakes.

8. Examine the exposed mantle-lobe which lined the valve just examined. Note its thickened margin. Lift its edge, and note that its attachment to the body-wall, of which it is an expansion, is along an irregular line. Commencing above and in front of the anterior adductor muscle, it passes down and around the ventral side of that muscle, skirts the protractor pedis muscle on its ventral side, and then rising, follows the line of the base of the gills backwards to the ventral side of the posterior adductor. Curving around this muscle, it rises upwards and backwards, and becomes continuous with the attachment of the other mantle-lobe in the region of the ligament. Its margin is free except above the exhalent siphon, where it joins with the margin of its fellow, leaving, however, the supplementary aperture of the supra-branchial chamber already described as being above and in front of the exhalent siphon. The margins of the mantle-lobes come in contact with each other, but are not united, below both siphons, and thus form their limits.

Turn up the mantle-lobe, bring its under surface to the surface of the water, throw if necessary a strong light upon it, and note the working of the cilia with which it is covered.

9. A pair of **Gills** are now exposed. They are ciliated lamellar structures lying between the foot and the mantle-lobe. Their anterior extremities reach about as far forward as the umbo; behind the foot the inner gills of opposite sides unite to form the floor of the supra-branchial chamber.

10. In front of the gills are the **Labial Palps,** a pair of richly ciliated triangular bodies, each of which is continuous

with its fellow of the opposite side around the front of the **Visceral Mass** which overlies the foot. Between the outer and inner labial pulps in front of the visceral mass is the mouth.

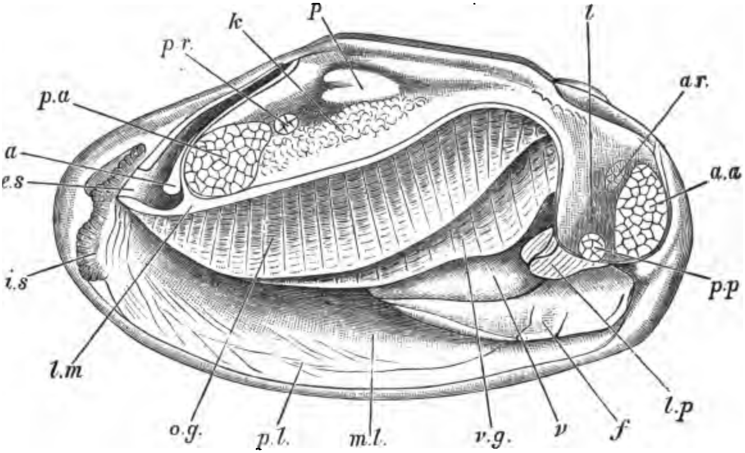

FIG. 152.—*Anodonta cygnea.* The animal is lying in one valve, and the mantle-lobe has been removed from the exposed side. *p*, pericardium; *k*, kidney; *p.r*, posterior retractor muscle; *p.a*, posterior adductor muscle; *a.a*, anterior adductor muscle; *a.r*, anterior retractor muscle; *p.p*, protractor pedis muscle; *a*, anus; *e.s*, exhalent siphon; *i.s*, inhalent siphon; *l.m*, attachment of mantle-lobe; *o.g*, outer gill-plate; *p.l*, pallial line; *m.l*, mantle-lobe; *v.g*, inner gill-plate; *v*, visceral mass; *f*, foot; *l.p*, labial palps; *l*, liver.

11. Occupying the anterior median portion of the branchial chamber is a body, the lower reddish portion of which is the muscular foot, the upper a mass of viscera consisting of the liver and sexual organs through which runs the digestive tract.

12. Turn the gills and mantle-lobe down again, and note in the dorsal region at about the middle of the length of the animal a transparent space, within which movements are seen. The cavity is the **Pericardium**, the moving body within is the **Heart.**

13. Make a detailed examination of the structure of a gill-plate. With the naked eye it can be seen that it is vertically and transversely striate. With a hand lens it is made evident that the vertical markings are of two kinds, thin narrow rods, the **Gill-filaments,** which are close together, and broader bars

which occur at wider intervals. Cut out a piece of either gill-plate, examine the cut edge, and note that it is composed of two lamellæ, which are united together by the wider vertical bars spoken of above, the **Inter-lamellar Junctions.**

14. In an excavated cell mount a small piece of the outer lamella of the inner gill with its outer side uppermost, and another with its inner side uppermost. Cover without pressure, and examine with a medium power.

Focus the surface of the first piece, and note that each gill filament is strengthened near its outer edge by two chitinous rods. Focussing a little deeper it can be seen that the inner edges of the filaments are concrescent laterally, leaving, however, small oval pores. At the edges of the filaments, and in the hollows around the pores, cilia are in incessant motion. In the inter-filamentar junctions is much fibrous tissue which forms the transverse markings of the gills. Examine the second piece for confirmation. If a piece of the gill-plate which has been kept in alcohol be allowed to dry extended, and then be examined with a hand lens, its structure can be made out with surprising distinctness.

There is a common axis for the pair of gill-plates on each side of the body. These axes run longitudinally on the dorsal side from near the labial palps back to the exhalent siphon. The posterior portion is free, the anterior is concrescent with the body-wall. Two descending rows of gill-filaments have their origin in each axis. By the inter-filamentar junctions the filaments are united laterally and form the outer lamellæ of the inner gill-plates, and the inner lamellæ of the outer gill-plates. At the free edges of the gill-plates they ascend parallel to their descending portions, and thus form the outer lamellæ of the outer gill-plates, and the inner lamellæ of the inner gill plates.

The edges of the outer lamellæ of the outer gill-plates are concrescent with the mantle-lobe along their whole length. The edges of the inner lamellæ of the inner gill-plates are concrescent with the visceral mass in its anterior portion, free near its posterior portion, and united with each other behind it. Between the free portions of their edges and the visceral mass the branchial communicates with the supra-branchial chamber.

15. Take a mussel hardened in chromic acid, and remove the mantle-lobes just below their lines of attachment. Insert a pair of scissors through the exhalent siphon into the supra-branchial chamber, cut along the attachments of the outer lamellæ of the outer gills, then just above the gill-axes, finally through the base of the foot and visceral mass, completely removing the dorsal portion of the body.

The bases of the gills are now exposed, and their relations to the gill-axes and to each other can be studied in detail. The inter-lamellar junctions are here very wide and can be well seen.

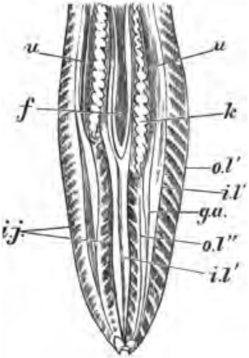

FIG. 153.—*Anodonta cygnea.* Floor of supra-branchial chamber. *f*, foot; *k*, kidney; *u*, ureter; *g.a*, gill-axis; *i.j*, inter-lamellar junctions; *o.l'*, *i.l'*, outer and inner lamellæ of outer gill-plate; *o.l''*, *i.l''*, outer and inner lamellæ of inner gill-plate.

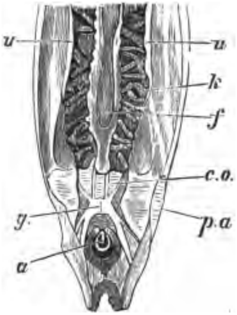

FIG. 154.—*Anodonta cygnea.* Roof of supra-branchial chamber. *a*, anus; *p.a*, posterior adductor muscle; *o.g*, olfactory ganglia; *c.o*, cerebro-olfactory connectives; *f*, foot; *k*, kidney; *u*, ureters.

16. The following should also be noted in this dissection:—On the inner side of the anterior portion of each gill-axis, close to and parallel with it, is a channel, the **Ureter**, which opens at its anterior limit by a narrow slit into the space between the lamellæ of the inner gill immediately in front of the free edge of the inner lamella. Pass a guarded bristle into it, turn up the animal, and note its point of emergence through the **Renal**

Aperture. Inside the ureters on each side are the **Kidneys (Nephridia)**, sacculated organs whose walls carry a mass of tubules. These overlie the spaces over the inter-lamellar junctions of the inner gill-plates, and in the dissection they, as well as the ureters, are divided longitudinally.

Examine now the under surface of the dorsal portion, that which formed the roof of the supra-branchial chamber. The remainder of the kidneys is now exposed to view. They are separated from each other in the middle line by a thin membrane only. On the outer side of the anterior portion of each is exposed a portion of the ureter. Pass a seeker back into each, and note that they lead back over the kidneys. Separate the tubules in the roof of the posterior portion of the kidney, and note a small irregular opening on the inner side which leads up into the ureter. Pass a guarded bristle through it into the ureter.

It has now been shown that the kidney communicates with the ureter, and the ureter with the exterior. It will be seen in a later dissection that the pericardium communicates with each kidney, so that the pericardium, which is the primitive body-cavity, communicates with the exterior through the kidney.

17. Take another hardened mussel, remove one valve, and pin out the other with the animal lying in it under water. Slit open the pericardium and remove as much of its wall as is necessary to expose the organs within it.

These are the **Heart** and the great vessels opening from it, the **Anterior** and **Posterior Aortæ**, with a portion of the **Rectum,** the terminal portion of the intestine. The heart consists of a median thick-walled muscular **Ventricle,** through which passes the rectum, and two thin-walled **Auricles** communicating laterally with the ventricles by wide slits with pouched lips which form valves.

18. Cut through the auricle on the exposed side, and note its communication with the ventricle. Slit open the ventricle, and note its internal fibrous muscular wall and the rectum running through it. Pass a seeker from the ventricle into the anterior aorta lying on the rectum, and into the posterior aorta lying

beneath the rectum. The ventricle is originally free from the rectum; during development it grows around it.

19. Cut through the rectum and aortæ near the anterior and posterior limits of the pericardium, also through the other auricle, and remove the whole. Take the opportunity of

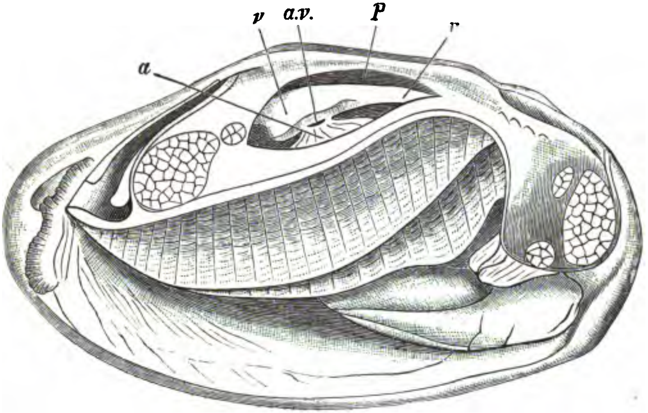

FIG. 155.—***Anodonta cygnea.*** The pericardium opened out. *v*, ventricle; *a*, auricle; *a.v*, auriculo-ventricular aperture; *p*, pericardium; *r*, rectum.

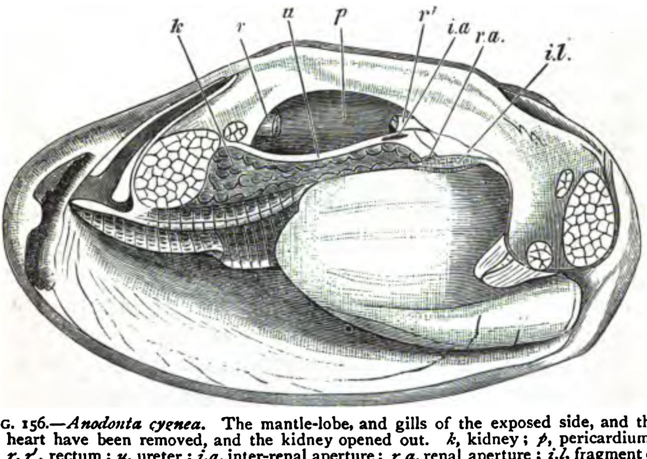

FIG. 156.—*Anodonta cygnea.* The mantle-lobe, and gills of the exposed side, and the heart have been removed, and the kidney opened out. *k*, kidney; *p*, pericardium; *r*, *r'*, rectum; *u*, ureter; *i.a*, inter-renal aperture; *r.a*, renal aperture; *i.l*, fragment of the inner lamella of the inner gill-plate.

examining the cut end of the rectum and noting that its floor is raised up into a longitudinal projection, the **Typhlosole.** Look in the floor of the pericardium in its most anterior portion for the slits—**Reno-pericardial Openings**—which communicate with the kidneys. In the median line of the floor of the pericardium is a large blood sinus, the **Vena Cava,** separated from the pericardium by but a very thin membrane. Open up this very carefully without injury to the renal organ on each side of it.

The **Blood** of the mussel is a colourless coagulable fluid holding in suspension nucleated amœboid corpuscles. Mount a drop of blood taken from a freshly killed animal, and examine with a high power.

The course of the blood-vessels is difficult to follow without injection. The venous system is mainly represented by irregular lacunar spaces into which the blood passes from the tissues. Shortly, the course of the circulation is this: By the contraction of the ventricle the blood is forced into the aortæ. The anterior aorta supplies the visceral mass and foot, the labial palps, anterior muscles, and the anterior portion of the mantle. The posterior aorta supplies the posterior muscles and the posterior portion of the mantle. It then collects in the lacunar blood-spaces, and from them it passes into the vena cava. From the vena cava it flows by a number of vessels through the kidneys and thence into large vessels lying in the gill-axes. From these vessels it passes into the branchial vessels and spaces lying in the inter-lamellar junctions, and from them by many openings which can readily be seen at this stage of the dissection to the expanded bases of the auricles. From the structure of the gills it is inferred that they are not nearly so effective for respiration as are the mantle-lobes.

20. Pass the end of a blow-pipe into the reno-pericardial opening, which is uppermost, and inflate the kidney and ureter. Slit up the delicate thin roof of the ureter which lies upon and to the outer side of the kidney, and, cutting through the mantle-lobe and gills, trace it to its external opening. Inflate again, and note by the bubbles passing through, the aperture by which the kidney communicates with the ureter. The two

ureters communicate with each other anteriorly by a wide slit, the **Inter-renal Aperture.**

21. Make a transverse section of a hardened mussel through the ventricle and determine the relations of the parts to each other. Other transverse sections should be made about a quarter of an inch apart from each other, and the section laid out in order beneath water or alcohol, and studied.

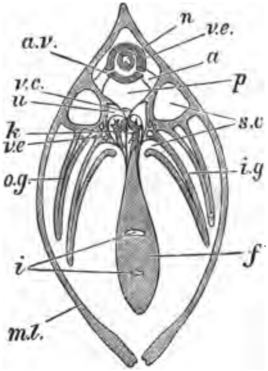

FIG. 157.—*Anodonta cygnea.* Transverse section through the heart. *n*, rectum; *v.e*, ventricle: *a*, auricle; *a.v*, auriculo-ventricular aperture; *p*, pericardium; *s.c*, supra-branchial chamber; *v.c*, vena cava; *u*, ureter; *k*, kidney; *o.g*, outer gill; *i.g*, inner gill; *i*, intestine; *f*, foot; *m.l*, mantle-lobe.

22. For the dissection of the digestive system a living mussel should be kept for a few hours in water containing finely divided carmine in suspension. This should be gently stirred occasionally to prevent it sinking to the bottom of the vessel. The animal should then be killed in hot water as already described, and kept in alcohol for a few days. On dissection the digestive tract can be readily followed by the carmine which it will contain. Remove first the mantle, gills, and labial palps of one side whilst the animal lies in one valve of the shell. Pass a seeker into the mouth and follow the seeker with a knife, removing the tissues as they become separated so as to get a longitudinal section of the whole tract. The following portions will be successively exposed :—

(*a*) A short vertical **Œsophagus.** This leads into

(*b*) An expanded **Stomach.** The stomach lies in the dark brown tissue of the **Digestive Gland,** the so-called **Liver,** which opens into the stomach by ducts.

(*c*) The **Intestine** opens from the floor of the stomach and passes downward and backward in the soft mass of the sexual organ. In this portion the typhlosole is well developed on the ventral side. The intestine then forms three loops, not remaining constantly in the median plane, and finally passes into (*d*) The **Rectum,** where the typhlosole is also well developed. The passage of the rectum through the pericardium has already been noted.

(*e*) The extremity of the rectum is the **Anus.** This opens in the roof of the supra-branchial chamber on the summit of a papilla close to the exhalent siphon. The posterior portion of the supra-branchial chamber is not unfrequently called the **Cloacal Cavity.**

23. Scrape the wall of the intestine, mount the scrapings in water, and examine with a high power.

They consist mainly of closely packed columnar epithelial cells, with broad ciliated free extremities and simple or divided filiform bases. A few unciliated cells appear to be secretory in function.

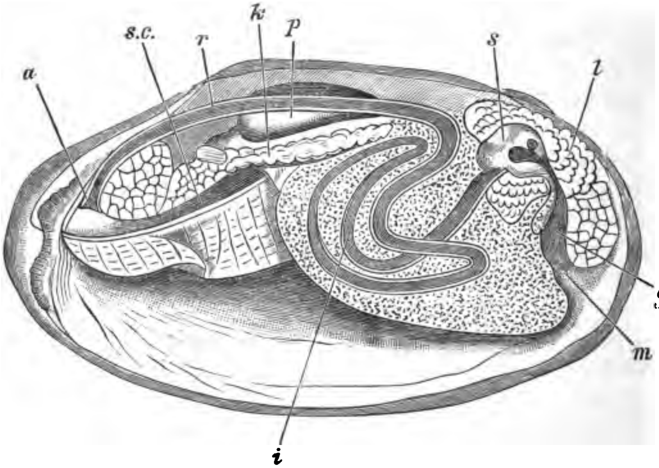

FIG. 158.—*Anodonta cygnea.* Median longitudinal section. *m*, mouth; *g*, guilet; *l*, liver; *s*, stomach; *i*, intestine; *p*, pericardium; *k*, kidney; *r*, rectum; *s.c*, supra-branchial chamber; *a*, anus.

24. The nervous system of the mussel consists of three pairs of nerve-ganglia connected by nerve-commissures, and giving off nerve-trunks.

The **Cerebral Ganglia** are a pair of triangular orange-coloured bodies, each lying very little below the surface between the protractor pedis and the anterior adductor muscles. Use a hardened mussel, break through the integument at this point, and the ganglion is exposed. From the cerebral ganglia the muscles in their vicinity, the labial palps, and the anterior portion of the mantle are supplied with nerves. From each cerebral ganglion arise three commissures, connecting it with its fellow of the opposite side and with the other ganglia.

The **Cerebral Commissure** arises from the anterior portion of the ganglion, and, passing behind the anterior adductor muscle and before the mouth, joins its fellow of the opposite side. Press away the muscle from the tissues behind it and the commissure will come into view.

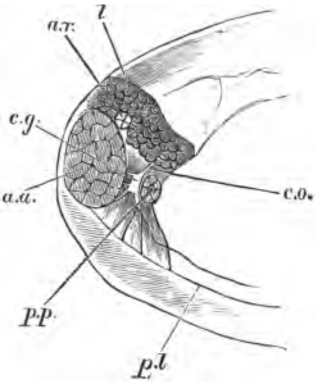

FIG. 159.—*Anodonta cygnea.* *c.g*, cerebral ganglion; *c.o*, cerebral-olfactory connective; *l*, liver; *a.a*, anterior adductor; *a.r*, anterior retractor; *p.p*, protractor pedis muscle; *p.l*, pallial line.

The **Cerebro-pedal Commissure** arises from the posterior inner angle of the ganglion, and passes straight to the **Pedal Ganglion** of its side, lying on the borders of the foot. To see this commissure, disconnect the ganglion from the cerebral commissure and from the nerves on its anterior and ventral sides.

The **Cerebro-olfactory Commissure** of each side arises from the dorsal angle of the ganglion. It runs near the surface, first through the liver, then through the sexual organ back to the inner border of the kidney. It may be easily exposed by following it up from the ganglion, breaking through the tissues overlying it with the point of a needle. These commissures connect the cerebral with the **Olfactory Ganglia** lying beneath the posterior adductor muscle.

The **Pedal Ganglia** lie between the muscular foot and the sexual organ. They are closely apposed, and may be most easily found by snipping off the ventral edge of the foot with a pair of scissors and then slowly tearing the halves of the foot apart until the orange-coloured ganglia come into view. They supply nerves to the foot and the visceral mass, and one small trunk passes from each to the **Auditory Sac,** containing a small **Otolith.** This lies a little behind and below the ganglion. It is not easily found, although with care and patience it may be exposed. The cerebro-pedal commissures are easily traced up to the cerebral ganglia.

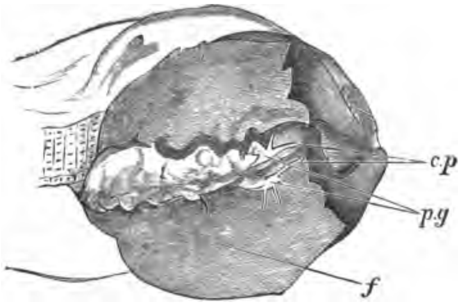

Fig. 160.—*Anodonta cygnea.* The foot *f* has been split in half, exposing the pedal ganglia, *p.g*, and the cerebro-pedal connectives, *c.p*.

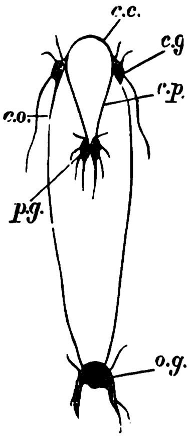

Fig. 161.—Nervous system of *Anodonta cygnea.* *c.g*, cerebral ganglia; *p.g*, pedal ganglia; *o.g*, olfactory ganglia; *c.c*, cerebral connective; *c.p*, cerebro-pedal connectives; *c.o*, cerebral-olfactory connective.

The **Olfactory,** or **Parieto-splanchnic Ganglia,** lie in the roof of the suprabranchial chamber beneath the posterior adductor muscle and close to the surface. They may be exposed by opening up the chamber in the manner already described, and removing the thin epidermis covering the ventral surface of the muscle. They are closely connected in the median line, and give off nerves to the muscles adjacent, and to the posterior portion of the mantle. The edges of the mantle bordering the siphons are especially well supplied.

25. The **Sexual Reproductive Organs (Gonads)** are situated above the foot and behind and beneath the liver. The mussel is diœcious, **Ovary and Testis** being found in different individuals. Each gonad is a racemose gland consisting of many blind diverticula, opening by its duct to the

exterior close to the renal aperture. In appearance the gonads resemble each other closely. If, however, a small piece be teased out—in normal salt solution, if fresh—and examined with a high power, the difference is plain.

The **Testis** will show, in winter and early spring, numbers of motile **Spermatozoa,** with rounded heads and long vibratile tails.

The **Ovary** at the same period will show **Ova** in various stages of development. The mature ovum is a spherical nucleated cell, with a dull glassy appearance.

The **Genital Aperture** is close to the renal aperture. The ova are ejected into the supra-branchial chamber and pass into the spaces between the lamellæ of the outer gill-plates, which function as brood-pouches. Here they are fertilized by spermatozoa introduced in the inhalent current, and here they pass through the first stages of development which result in the production of a curious larval form, the **Glochidium,** formerly believed to be a parasite of the mussel. The glochidium is triangular. It has two valves, each provided with teeth and a sharp curved beak, a single adductor muscle, and a long thread, the **Byssus,** by which it is attached to the gill of the parent. The development is slow. It is not until autumn that the glochidium quits the parent and swims freely for a time by the alternate opening and closing of the valves

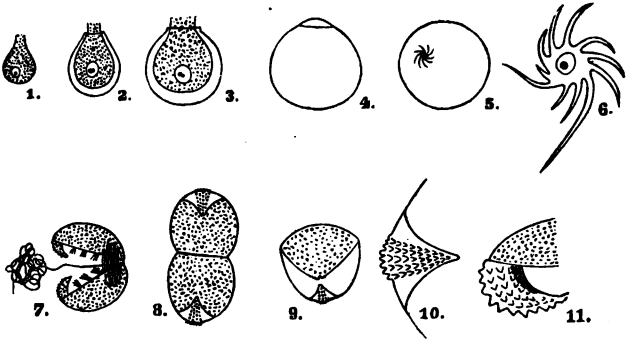

FIG. 162.—Mussel. 1–3, young ova; 4, ripe ovum; 5, ovum showing spiral star; 6, spiral star much magnified; 7, glochidium with byssus; 8, 9, glochidium shells; 10, 11, beak of glochidium much magnified. (Morgan.)

of its shell, until it comes in contact with a fish in whose fin it hooks its curved beak. Here it encysts itself and passes through the remaining stages of its development. Finally it drops to the bottom of the water and assumes the form and habits of its parent mussel.

CHAPTER XVI.

THE RIVER CRAYFISH (ASTACUS FLUVIATILIS).

THE common river crayfish is found in the shallow parts of rivers and other bodies of fresh water over the whole of England and Ireland; in some places in immense numbers. It is carnivorous and even cannibal in its propensities. Lurking under stones or in burrows excavated by itself in the soft banks of the river, it seizes on its unsuspecting prey by its powerful chelæ, tears it in pieces, and conveys it to its mouth. The animal attains a length when full grown of five or six inches. It is brownish in colour, with very often a tinge of green in it, and is in structure very similar to, though smaller than, the edible lobster, *Homarus vulgaris*, of our seas, to which it is so nearly related that in almost all respects the following description will apply equally well to the lobster. The student should be careful, however, if he uses lobsters,

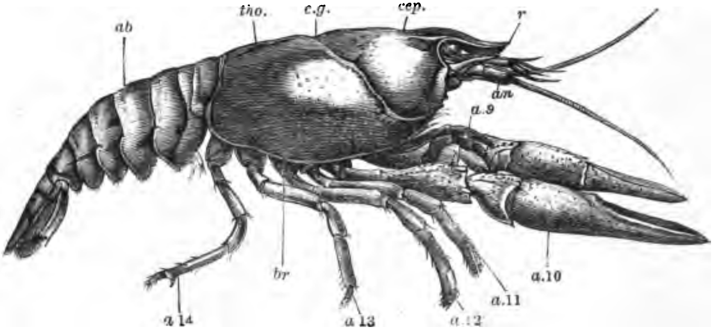

FIG. 163.—The crayfish. *r*, rostrum; *cep*, cephalic portion; *tho*, thoracic portion of the cephalothorax; *c.g*, cervical groove; *ab*, abdomen; *br*, branchiostegite; *an*, antenna; *a* 9, 10, 11, 12, 13, 14, appendages.

to get them unboiled, and not the familiar red specimens of the fishmonger's shop.

The whole body is covered externally by a tough cuticle, which everywhere, except in places where motion of parts on one another takes place, is hardened by infiltration of carbonate and phosphate of lime. The cuticular covering is continued at the mouth and anus into the alimentary canal, which it wholly lines except a very short portion, the mid-gut. During the first year of the animal's life, when growth is actively going on, the cuticle, which has no power of growth, is cast off two or three times, a new one being secreted in a few days after each **Ecdysis.** Afterwards ecdysis takes place about once a year. In its soft and defenceless condition the crayfish falls an easy prey to its enemies, from whom it endeavours to escape by going into hiding until its new shell is hard enough to be a sufficient protection. The body of the crayfish has great capability for repairing injuries. If a limb is injured it is cast off and a new one grows in its place.

The sexes are separate, the testis being in one animal, the ovary in another. They are also to be distinguished externally by certain **Secondary Sexual Characters**; the female is broader in the abdomen than the male, and certain of the abdominal appendages are different in the two sexes.

Live crayfish should be obtained and kept, preferably in an aquarium with glass sides through which they can be watched. The animal moves about slowly by walking with its four pairs of ambulatory legs. Alarm it by suddenly thrusting something at it, and the animal darts backward by the sudden flexion of its abdomen. Turn it over on its back, and mark the motion of its swimmerets (abdominal appendages) in reversing its position. Touch an eye, and note the mobility of the eye-stalk. Set free from a pipette, near the bases of its ambulatory legs, water holding colouring matter, such as finely divided carmine or indigo, in suspension, and note the current sucked in under the carapace and coming out from the cervical canal not far behind the mouth. Feed it with small animals or meat, and note the prehension and mastication of the food.

The crayfish will live fairly well in captivity if well looked after. Professor Howes says that it may be kept alive for some days in a damp atmosphere if fed on sopped bread. It is best, however, not to feed it for a few days previous to dissection. It may be killed, almost instantaneously, by dropping it into boiling water, but it should be at once withdrawn. If not dissected immediately after death it should be put into alcohol, and for almost all purposes of dissection it is better for having been in alcohol for a few days.

External Characters.

1. Take a dead crayfish and note the following:—The body is bilaterally symmetrical. It is plainly separable into two portions—an anterior portion called the **Cephalothorax**, covered by a continuous piece of the **Exoskeleton**, the **Carapace**, and a posterior portion, the **Abdomen.** This latter is segmented and each segment is movable, with some amount of freedom on its neighbours.

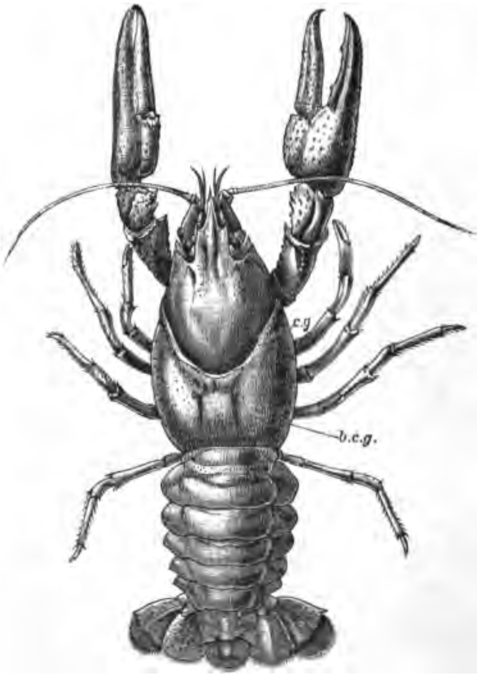

FIG. 164.—The crayfish. Dorsal surface. *c.g*, cervical groove; *b.c.g*, branchio-cardiac groove.

The cephalothorax is divided externally by a deep groove, the **Cervical Groove,** into an anterior **Cephalic** and a posterior **Thoracic** portion. The cephalic groove, which is transverse on the dorsal surface, runs forward on each side and terminates just below the insertion of the **Antennæ.** The carapace is prolonged in front beyond the eyes to terminate in a sharp beak, the **Rostrum.** The thoracic portion has two longitudinal grooves on it—the **Branchio-cardiac Grooves,** which mark off a median dorsal **Cardiac** portion from two lateral portions, the **Branchial** or **Gill Chambers.** These latter are bordered on the outer sides by overhanging portions of the carapace, the **Branchiostegites,** whose free edges below are thickly fringed with setæ. The thoracic portion of the carapace is a backward growth of the cephalic portion.

The abdomen is formed of seven segments, so articulated that each has a range of motion upon its neighbour equal to an angle of about 30°. The consequence is that the whole abdomen can be so flexed that the terminal segment can pass from a straight line with the body through an angle of 180°. The limit of extension is the straight line, which is the body-axis.

2. Examine the third or fourth abdominal segment and note—

The hard convex shell, the **Tergum,** covering its dorsal surface. This is prolonged downward at the sides into the **Pleura,** which are projecting pieces fringed with setæ. Turn the animal on its back and examine the ventral surface. Here the hard portion of the segment, the **Sternum,** is narrow. Like all the other segments, except the last, it carries a pair of appendages, hinged on by uncalcified cuticle—**Arthrodial Membranes**—and capable of movement in one plane only. Between the appendages and the pleura are short calcified bars, the **Epimera.** The anterior portion of the tergum is smooth, and is overlapped in extension of the abdomen by the posterior portion of the preceding tergum.

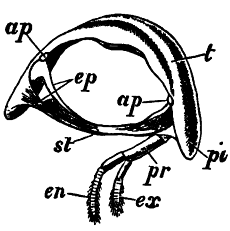

FIG. 165.—Fourth abdominal segment of the crayfish. *t*, tergum; *st*, sternum; *e.p*, epimeron; *pl*, pleuron; *ap*, articular peg; *pr*, protopodite; *en*, endopodite; *ex*, exopodite. One appendage has been removed.

3. The articulation of the third with the fourth segment should be studied in detail, and in order to do this the segments should be removed. Slip a sharp knife underneath the tergum, and move it from side to side to sever the connecting membrane. Next free the sterna from each other, and then slide off the freed portions of the exoskeleton. The two segments are connected all round by a tough uncalcified cuticle, which is loose on the dorsal side during extension, and on the ventral side during flexion of the abdomen. This cuticle restricts the movements of one segment on the other. The anterior portion of the tergum of the fourth segment is smooth, and is overlapped by the posterior portion of the tergum of the third segment. The former bears, just at the origin of the pleuron on each side, a small rounded peg which fits into the angle formed by the epimeron and the sternum of the third segment. There is a similar but smaller protuberance on the inner side of the posterior margin of the third segment, close to the same angle, which works in the groove just above the peg of the fourth segment. The anterior outer peg of the fourth segment thus articulates in the hollow between the epimeron and posterior inner peg of the third segment, and by this means lateral movement is prevented. The articulations of the other segments should be carefully examined, when differences will be found. Thus the pleura of the second segment overlap those of the first and third, and the posterior edge of the carapace fits into a deep groove on the anterior edge of the first abdominal segment, an arrangement which effectually prevents this segment being telescoped into the cavity of the thorax.

There is every reason to believe that the cephalothorax is also formed of coalesced segments homologous with those of the abdomen. The evidence for this is found on examination of the sternal region. The last thoracic sternum is distinct, those in front of it are fused together. The fourteen paired appendages, however, mark out the number and positions of the fused segments.

4. Examine in detail one of the appendages of the third abdominal segment.

Each is composed of a proximal basal portion, the **Protopodite,** which is two-jointed, and two distal portions, the **Endopodite** and **Exopodite,** each of which is many-jointed and fringed with hairs. The exopodite is on the outer side.

The last abdominal segment, the **Telson,** is imperfectly hinged transversely. It bears no appendages, and has on its ventral surface the slit-like **Anus,** which can be closed by the approximation of its sides.

5. Remove carefully all the appendages of one side, beginning with the twentieth; examine each one in detail, and mount them on a card for future reference.

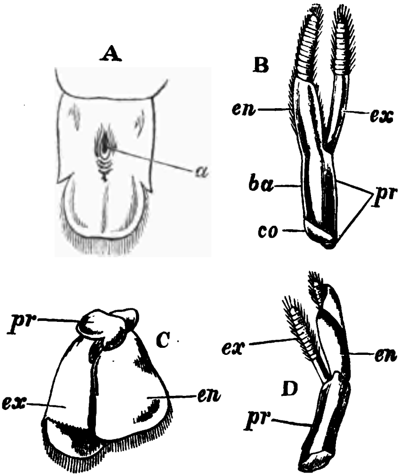

Fig. 166.—Abdominal appendages of the crayfish. A, telson; B, fourth abdominal appendage; C, appendage of the twentieth segment; D, second abdominal appendage of the male. *a*, anus; *pr*, protopodite; *ex*, exopodite; *en*, endopodite; *co*, coxopodite; *ba*, basipodite.

i. Six pairs of **Abdominal Appendages.**

Twentieth. The protopodite is small and undivided, the exopodite is flat with a transverse hinge; the endopodite is also flat and large, but unhinged.

Nineteenth, eighteenth, and seventeenth. These are small swimmerets. Each consists of a two-jointed protopodite, the proximal shorter joint being the **Coxopodite,** the longer distal joint the **Basipodite,** together with a longer endopodite and a shorter exopodite, the two latter being many-jointed.

Sixteenth. In the female this is like the seventeenth, or it may be very imperfectly developed.

In the male the long proximal joint of the endopodite is lengthened at its free extremity, and is there spoon-shaped, with one edge rolled on itself like a scroll. This is a secondary sexual character, which subserves fertilization.

Fifteenth. In the female this is always very rudimentary, and may even be absent.

In the male it is an undivided rod, with a spoon at its free end.

ii. There are eight pairs of **Thoracic Appendages.** The fourteenth to the eleventh inclusive are ambulatory legs; the tenth carry the large prehensile **Chelæ,** the ninth to the seventh inclusive are foot-jaws — **Maxillipedes** —and are used for passing food from the chelæ to the jaws.

Remove the branchiostegite on that side, cutting along just below the branchio-cardiac groove and the cervical groove, taking care to keep the point of the scissors close to the carapace.

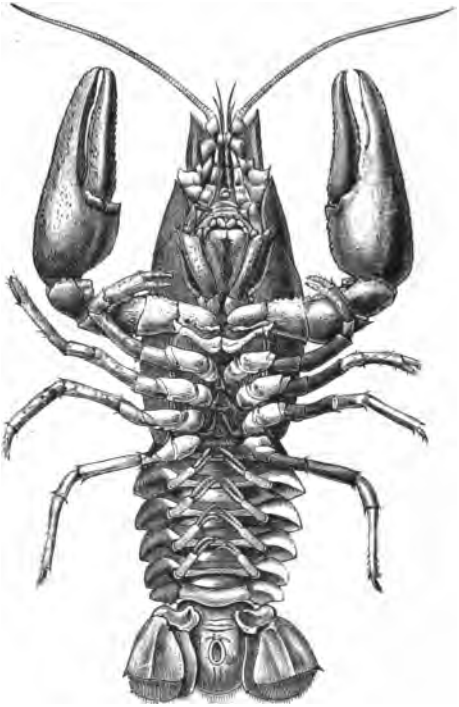

FIG. 167.—The crayfish; ventral surface.

Fourteenth. The protopodite consists of short coxopodite and basipodite. There is no exopodite, and the endopodite consists of five joints, named, from the basipodite outwards, the **Ischiopodite, Meropodite, Carpopodite, Propodite,** and **Dactylopodite.**

In the male the **Genital Pores,** terminations of the **Vasa Deferentia,** are on the summits of small papillæ, situated on the inner sides of the coxopodites of the fourteenth segment.

Thirteenth. This is like the fourteenth, with the exception that the coxopodite carries a flat plate, the **Epipodite,** within the branchial chamber, which bears a **Gill.** The coxopodite also carries a bunch of setæ.

Twelfth. This is like the thirteenth, with the exception that the propodite is continued beyond the articulation of the dactylopodite, so as to form a pair of pincers, which assist in capturing prey.

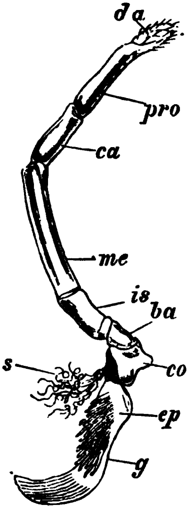

Fig. 168.—Twelfth appendage of the crayfish. *ep*, epipodite; *g* gill; *s*, setæ; *co*, coxopodite; *ba*, basipodite; *is*, ischiopodite; *me*, meropodite; *ca*, carpopodite; *pro*, propodite; *da*, dactylopodite.

In the female, the **Oviducts** open on the coxopodites of the twelfth segment.

Eleventh. This is like the twelfth.

Tenth. This is like the eleventh, but very much larger and more powerful. The basipodite and ischiopodite are ankylosed together.

Ninth. This is the third maxillipede. The protopodite consists of very short coxopodite and basipodite, the former bearing a gill and setæ, the latter a small exopodite and a large five-jointed endopodite.

Eighth. The second maxillipede is smaller and flatter than the third. It has the same number of joints.

Seventh. This is the first maxillipede. The coxopodite and basipodite form a thin bilobed plate. The epipodite is large and thin, and bears no gill. The endopodite is small, the exopodite a little larger.

iii. The **Cephalic Appendages** are six in number. The sixth and fifth are **Maxillæ,** the fourth are **Mandibles,** the third **Antennæ,** the second **Antennules,** the first **Eye-stalks.**

Sixth. This is the second maxilla. Both coxopodite and basipodite are large flat bilobed plates. The endopodite is small and unjointed; the exopodite and endopodite are fused together and form a large plate, the **Scaphognathite.**

Fifth. The first maxilla is small. The two joints of the protopodite are thin plates. The endopodite is also a flat plate, and there is no exopodite.

In front of the first maxilla is a narrow plate of cuticle fringed on the inside with hairs. This is an outgrowth of the **Metastoma,** which borders the mouth.

Fourth. This is the mandible. Before removing it note its possible side-to-side motion. The protopodite, or jaw, is much developed, and is very hard and bony. On its inner edge it is toothed like a saw, and this toothed edge works against the similar edge of its fellow. The endopodite is three-jointed and lies in the groove of the jaw.

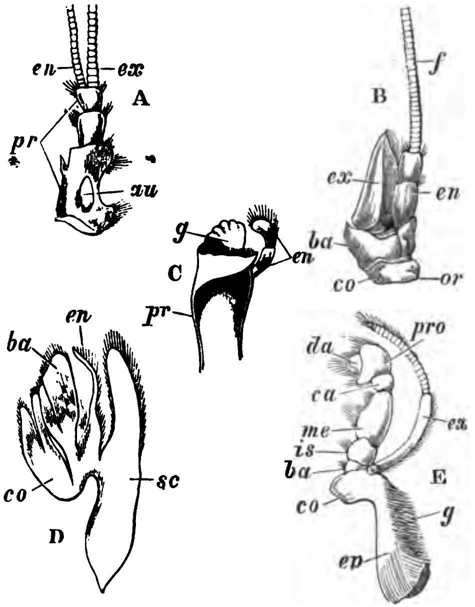

FIG. 169.—Crayfish appendages. A, antennule; *au*, auditory opening; B, antenna; *or*, renal opening; *f*, feeler; C, mandible—the distal joint of the endopodite normally lies in the groove *g*; D, second maxilla; *sc*, scaphognathite; E, second maxillipede. Other letters as before.

Third. This is the antenna. The coxopodite has on its ventral surface a small light-coloured projection, on the posterior side of which is the aperture of the **Renal Organ.** The basipodite bears endopodite and exopodite. The former consists of two proximal joints, and a long many-jointed feeler. The latter is an undivided triangular pyramid, with a very sharp apex directed forward. It is called the **Scaphocerite.**

Second. This is the antennule. The protopodite consists of three joints. On the dorsal side of the proximal joint is the opening of the **Auditory Organ,** covered with short setæ. The endopodite and exopodite are many-jointed filaments.

First. This is the eye-stalk. It consists of a two-jointed protopodite, carrying the eye at its free extremity.

6. The **Gills,** or **Branchiæ.**

Six pairs of branchiæ, the **Podobranchiæ,** have been already described, attached to the epipodites of the appendages from the second maxillipede to the third ambulatory leg inclusive. Turn towards the ventral surface all the branchiæ on a side from which the branchiostegite has been removed, and return them in succession, noting their points of attachment in each case.

(*a*) One **Pleurobranchia** attached to the epimeron above the fourth ambulatory leg. In front of this are two or three rudimentary pleurobranchiæ represented by small bars.

(*b*) Two **Arthrobranchiæ** attached to the articular membranes of each appendage from the third ambulatory leg to the third maxillipede inclusive, and one to the second maxillipede, making eleven in all.

(*c*) Six **Podobranchiæ,** already described.

There are thus eighteen pairs of branchiæ in all. The blood circulates through the branchiæ, and in them is separated from the surrounding water only by an extremely thin membrane. Through this the gases in the blood and water diffuse, the net result being a loss of carbon dioxide from the blood to the water, and a gain of oxygen from the water to the blood. The respiratory current of water under the branchiostegite over the gills has been described. If that portion of the branchiostegite which covers the scaphognathite be removed from a living crayfish, the latter will be seen sculling out the water, and thus keeping up the current through the cervical canal.

General Internal Structure.

One crayfish should be used to gain a general knowledge of the internal anatomy before the systems are studied in detail. Use an animal which has lain in alcohol for some days.

7. Pin out the crayfish in water, dorsal side uppermost, and with a pair of scissors cut away the exoskeleton from that side, inserting the point of the scissors first underneath the posterior ends of the branchio-cardiac grooves and cutting forward to the rostrum. Remove this piece of the carapace, and, turning the animal round, continue the longitudinal cuts to the telson, removing the terga as they are freed. Be extremely careful that the point of the scissors does not injure any of the organs lying below the exoskeleton. Cut now through the membranes covering the thorax, reflect or remove them, and thus expose the **Pericardial Sinus.** In this note—

(*a*) The **Heart,** a polygonal organ with thick muscular walls. It is held in position by three pairs of fibrous bands, the **Alæ Cordis,** which pass from it to the walls of the sinus. On its exposed dorsal side will be seen two oblique valvular slits, called **Ostia**; through these and two other pairs not apparent in this position it receives blood from the sinus.

(*b*) The blood passes out from the heart through **Arteries.** These are difficult to see in the fresh state, but in alcohol material they are very plain. From the anterior end of the heart passes a median **Ophthalmic Artery,** which goes straight forward in the median line over the stomach, and a pair of **Antennary Arteries** arising close to the origin of the ophthalmic artery and passing forward one on each side of the stomach. From the posterior end of the heart arises the **Dorsal Abdominal Artery,** passing backward in the middle line and lying on the intestine.

(*c*) Cut this through a short distance from the heart, reflect it over the heart, and see the origin of another large artery, the **Sternal Artery,** which goes vertically downward, sometimes to the right, sometimes to the left of the intestine, to the ventral side of the body.

(*d*) Cut through the sternal artery close to the heart, cautiously lift up the posterior end of the heart, and reflect it forward until the points of attachment of another pair of arteries, the **Hepatic Arteries,** are seen. These arise below and somewhat behind the origin of the antennary arteries.

(*e*) Note now two other pairs of ostia, one pair ventral, the other lateral. Remove the heart from the pericardial sinus and keep it for more detailed study. There is now exposed in the middle line—

(*f*) If the animal is a male, the **Testis**, a large organ bilobed in front, but with a single lobe behind. From about the middle, on each side, arises a **Vas deferens**, a very long convoluted tube which opens to the exterior on the coxopodite of the fourth ambulatory leg.

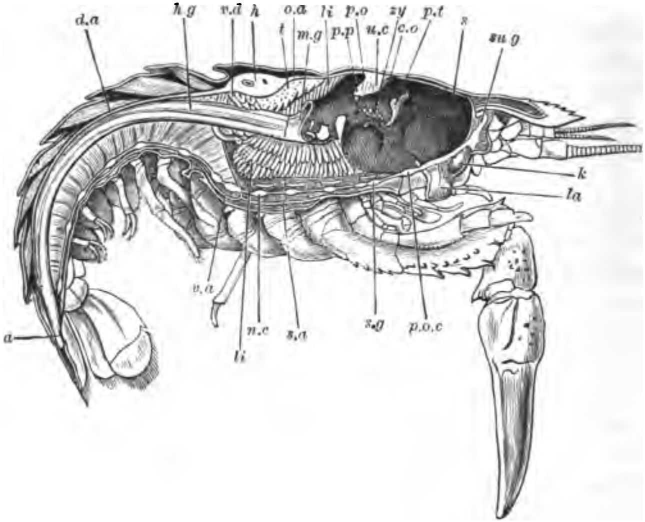

FIG. 170.—Median longitudinal section through a male crayfish. Natural size. *a*, anus; *c.o*, cardiac ossicle; *d.a*, dorsal abdominal artery; *h*, heart; *h.g*, hind-gut; *k*, kidney; *la*, labrum; *li*, liver; *m.g*, mid-gut; *n.c*, nerve chain; *o.a*, ophthalmic artery; *p.o*, pyloric ossicle; *p.p*, prepyloric ossicle; *p.t*, pterocardiac ossicle; *p.o.c*, para-œsophageal connective; *s*, stomach; *su.g*, supra-œsophageal ganglion; *s.g*, sub-œsophageal ganglion; *s.a*, sternal artery; *t*, testis; *v.d*, vas deferens; *u.c*, urocardiac ossicle; *v.a*, ventral abdominal artery; *zy*, zygocardiac ossicle. The right para-œsophageal connective has not been removed.

(*g*) If the animal is a female, the **Ovary**, very similar in shape to the testis. From the ventral side of each half arises a short wide tube, the **Oviduct**, not visible at this stage, which opens to the exterior on the coxopodite of the second ambulatory leg.

(*h*) To the right and left of the sexual organs, and extending beyond their anterior limits, are the lobes of the **Digestive Gland,** or **Liver,** a profusely branched organ of a dark colour.

8. Anterior to the thorax, the cephalic cavity is mainly occupied by the **Stomach,** a large organ which is only separated from the carapace by the muscles attached to it. Remove the muscles from the roof of the stomach, noting their points of origin and insertion. The chief are—

(*a*) The **Anterior** and **Posterior Gastric Muscles,** having their origins in the carapace and inserted, the former on the **Cardiac Ossicle,** a broad transverse bar crossing the middle of the dorsal side of the stomach, the latter on the **Pyloric Ossicle,** a similar but narrower bar posterior to the cardiac ossicle. From the cardiac to the pyloric ossicle extends the **Cardio-pyloric Muscle.** On each side note the broad ends of the **Mandibular Muscles,** which have their origin in the carapace above, and are inserted on the mandibles.

(*b*) The roof of the stomach is now exposed, and it is seen to be a large chitinous bag, transparent over great part of its surface, but ossified and opaque in parts.

9. Posterior to the thorax is the **Abdomen.** Note on each side, immediately beneath the terga of the abdominal segments, a long and powerful muscle, the **Extensor Abdominis,** running the whole length of the abdomen and passing forward into the thorax to its origin in the body-wall. Remove this muscle, and note beneath, in the middle line, the dorsal abdominal artery lying on the intestine.

10. Nearly the whole length of the digestive tract is now exposed. Three well-defined regions can be distinguished.

(*a*) The **Stomatodœum,** or **Fore-gut,** consisting of the **Œsophagus** and **Stomach.** The stomach is readily seen to consist of a larger anterior portion, the **Cardiac Chamber,** and a smaller posterior portion, the **Pyloric Chamber,** the two being separated by a constriction. The chitinous lining of the fore-gut is continuous with the chitinous external covering of the body.

(*b*) The **Mesenteron,** or **Mid-gut.** This is very short, and shows a dorsal dilatation, the **Cæcum.** The wall of the mid-gut has no chitinous lining. On each side of it are the openings of the **Bile-ducts** which pour into the digestive tract the secretion of the digestive gland (liver).

(*c*) The **Proctodœum, Intestine** or **Hind-gut,** runs straight from the mid-gut to the **Anus.** It has a chitinous lining, and its inner surface shows, even from the outside, white ridges, which wind round it in a steep spiral and are covered with white papillæ.

11. Cut through the bile-ducts nearly close to the mid-gut and remove the liver on both sides. Cut through the œsophagus close to the stomach, being extremely careful not to injure any of the nerves around the gullet, and remove the whole digestive tract. Keep this for more detailed study.

(*a*) On the floor of the cavity thus laid clear note the very powerful **Flexor Abdominis Muscles** originating in the body-wall, and passing back to their attachments in the sterna of the abdominal segments. In the angle where they meet passes down

(*b*) The **Sternal Artery** to the **Sternal Sinus,** at the point of entrance to which it gives off a branch. The main artery runs forward in the sternal sinus, the branch, which is the **Ventral Abdominal Artery,** runs backward to the telson.

(*c*) In the anterior portion of the cephalic cavity, on each side, is a large thin-walled sac. These are the **Ureters** which open to the exterior on the basal joints of the antennæ. Each ureter collects the secretion of the **Renal Organ (Kidney,** or **Green Gland),** a light-greenish glandular mass lying beneath it.

12. Remove the flexor abdominis muscles down to the telson. By this means the abdominal portion of the nerve-chain will be exposed. In the thorax the nerve-chain will be seen entering the sternal sinus together with the sternal artery, the latter passing below the nerve-chain between two of the connectives.

The sternal sinus is formed by the ingrowth of calcified cuticle from the sterna and inter-articular bars of the thoracic somites.

Cut away very carefully the roof of the sinus and so expose the thoracic portion of the nerve-chain. Remove carefully the sacs of the green glands, and the whole nerve-chain is now visible. Trace it from before, backwards, and note the following parts :—

(*a*) A large **Pre-œsophageal Ganglion** in the middle line just below and between the bases of the eye-stalks. From this nerves can be readily traced entering the eye-stalks, antennules, and antennæ.

(*b*) Leaving it behind, and passing backward, is a pair of large connecting nerve-trunks, the **Para-œsophageal Connectives**, passing one on each side of the œsophagus. On each connective is a ganglionic swelling by the side of the œsophagus, from which nerves are given off to the œsophagus and other parts.

(*c*) Another pair of much smaller nerves leaves the pre-œsophageal ganglion, and unites in front of the œsophagus with two pairs of nerves from the para-œsophageal ganglionic swellings to form a single trunk. This passes upwards as the **Anterior Visceral Nerve**, and supplies the stomach.

(*d*) Immediately behind the œsophagus the para-œsophageal connectives are connected by a narrow transverse commissure. Further back they unite to form the **Post-œsophageal** ganglion, from which nerves supply the appendages of the segments from the fourth to the eighth inclusive.

(*e*) Six **Thoracic Ganglia** follow, the first immediately behind the post-œsophageal ganglion, the next four with wider intervals between them. They are united by paired connectives which are, however, in close contact with each other except between the fourth and fifth, where the sternal artery passes between them. The fifth and sixth are close together. Each ganglion supplies its own segment with nerves.

(*f*) There are six **Abdominal Ganglia**, also united by paired connectives. The sixth, which is the largest, supplies the telson as well as the twentieth segment.

(*g*) The sixth abdominal ganglion also gives off a nerve, the **Posterior Visceral Nerve**, which turns forward and runs along the surface of the intestine.

13. Remove the nerve-chain, and thus expose the sternal and ventral abdominal arteries.

The Respiratory System.

14. Examine one each of the three kinds of branchiæ.

(*a*) The **Pleurobranchiæ** and **Arthrobranchiæ** consist of simple hollow **Branchial Filaments,** in which the blood is aërated, attached to the outer side of a central axis, in which are afferent and efferent blood-vessels which communicate with the cavities of the branchial filaments.

(*b*) The **Podobranchiæ** are not so simple in structure. The epipodite, to which each is attached, is long, corrugated over its distal surface, and folded on itself. The outer surface and edge carry the branchial filaments.

The Circulatory System.

15. The blood, aërated in the branchiæ, passes through channels between the epimera in the walls of the thoracic cavity into the pericardial sinus, and thence through the six pairs of ostia into the heart. The contractions of the heart drive the blood into the arteries, the valvular lips of the ostia preventing its return to the pericardial sinus, and by the arteries it is carried all over the body.

The ophthalmic artery supplies the eyes and other portions of the fore part of the head; the antennary arteries supply the stomach and muscles adjacent to it, the kidneys, and the antennæ; the hepatic arteries supply the liver; the superior abdominal, the dorsal region of the abdomen; the sternal artery gives off a branch to the sexual organs, and supplies also the sternal region of the thorax and the appendages from the last thoracic up to the mouth; and the ventral abdominal artery supplies the ventral side of the abdomen.

The arteries divide and subdivide into smaller and smaller tubes, the smallest of which are called **Capillaries,** and from the capillaries it floods the tissues to which they are supplied. In the vertebrate animals the capillaries are united again into **Veins,** which return the blood to the heart. In the crayfish there are no veins, but from the various tissues the blood passes into irregular spaces (sinuses) in various parts of the body, and eventually finds its way to the sternal sinus, whence it passes to the branchiæ.

The blood is a colourless fluid holding in suspension colourless amœboid corpuscles.

The Digestive System.

16. The food, which is seized and torn by the chelæ, is passed to the jaws, whose side-to-side motion further reduces it. The mouth is bordered in front by a flat plate, the **Labrum,** behind by the metastoma. The gullet is short, and during life is constricted so as to close the passage except when food is passing.

Within the stomach is another apparatus, the **Gastric Mill,** for grinding the food. Examine the stomach first from the outside, then cut away the anterior and ventral portions and examine the inside, and finally make a vertical longitudinal section through the gastric mill.

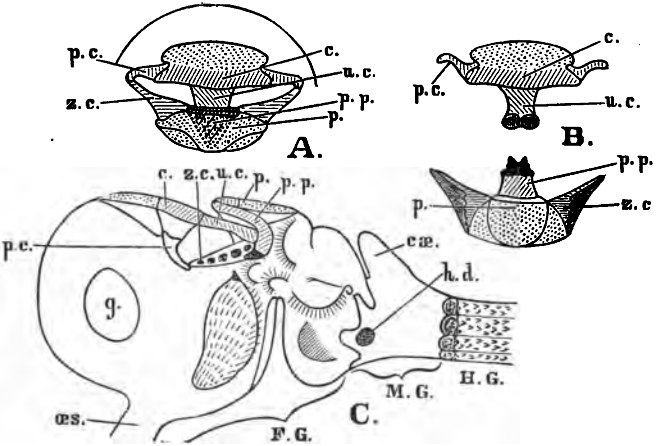

FIG. 171.—Stomach of crayfish. A, the ossicles from above; B, from below; C, longitudinal section. *c*, cardiac ossicle; *cæ*, cæcum; *g*, position of gastrolith; *h.d*, duct of digestive gland; F.G, fore-gut; M.G, mid-gut; H.G, hind-gut; *œs*, œsophagus; *p*, pyloric ossicle; *p.c*, ptero-cardiac ossicle; *p.p*, prepyloric ossicle; *u.c*, urocardiac ossicle; *z.c*, zygocardiac ossicle. (Morgan.)

This is formed mainly of four median, and two pairs of lateral ossicles. The **Cardiac Ossicle,** whose posterior portion is strongly calcified, is connected with the **Pyloric Ossicle** by two other median ossicles, both of which slope downwards and backwards to meet at an acute angle below and behind the pyloric. The more anterior, the **Urocardiac,** is attached to the cardiac in front; the more posterior, the **Prepyloric,** is attached to the pyloric and carries below, where it meets the urocardiac, a large, hard, bifid tooth. The contraction of the anterior and posterior gastric muscles separates the cardiac and pyloric ossicles, and by so doing depresses and pushes forward this tooth. Hinged to the cardiac on each side is a small lateral ossicle, the **Pterocardiac,** and to the pyloric on each side another, the **Zygocardiac.** Each zygocardiac bears on its inner edge a row of teeth, which are situated below and in front of the prepyloric tooth. The same cause which depresses and advances the prepyloric tooth brings the zygocardiac teeth together in the middle line, and these three grinding surfaces act in such a way that the prepyloric tooth forces the food between the toothed margins of the zygocardiacs. The student should imitate these movements by pulling the cardiac and pyloric ossicles apart.

During the summer months a deposition of lime salts takes place in two flat plates on the sides of the stomach. These are **Gastroliths.** They disappear during ecdysis.

At the entrance of, and in the pyloric chamber, there are plates fringed with coarse hairs, which act as strainers and prevent any but finely comminuted food from entering the mid-gut.

The chitinous layer is not continued over the inner surface of the mid-gut. Here the lining membrane is epithelial. Into it on each side opens the duct of the digestive gland. The secretion of this gland is a yellow fluid containing a ferment which converts insoluble proteids into peptones, another which converts starch into sugar, and in addition it emulsifies fats. It thus fulfils the functions of the secretions of the stomach, liver, and pancreas of higher animals.

The Excretory System.

17. Nitrogenous waste is excreted by the kidneys or green glands. The gland itself is an extremely long convoluted tube, the end of which, the ureter, is much expanded and opens to the exterior, as already described. The secretion of the kidneys holds large quantities of uric acid in solution. Pass a bristle tipped with sealing-wax into the sac of the gland from its opening on the basal joint of the antenna.

Organs of Special Sensation.

18. **The Eye.** Its position has already been noted. Cut thin surface sections from the outer transparent limiting layer, the **Cornea,** and note under the microscope that it is divided up into, at the sides square, and in the centre hexagonal, facets. Halve the eye longitudinally, and examine with a hand lens the structures lying immediately beneath the cornea. Tease out these structures, in normal salt solution if fresh, or in alcohol if alcohol material; examine with low and high powers of the microscope, and follow as far as possible the following description :—

(*a*) The cornea is a continuation of the cuticular covering of the body. It is divided up into facets, each of which is approximately a plano-convex lens, the slight convexity being on the inner side.

(*b*) Each facet of the cornea is at the base of a pyramid, all the **Crystalline Pyramids** lying along the radii of the sphere of which the eye is a segment. The outer ends of the crystalline pyramids are optically isolated from each other by pigment.

(*c*) The narrow apices of the crystalline pyramids widen out again into fusiform structures, which are striated transversely and are on that account called **Striated Spindles.** These are also imbedded in pigment.

(*d*) These are succeeded by the fibres and ganglionic cells of the optic nerve, which also lie in pigment.

From this description it will be seen that the eye of the crayfish is compound. The nature of vision by such an eye is not well understood. From the structure of the crystalline pyramids, it is clear that only rays of light which are parallel or nearly parallel to the axis of a pyramid can reach its apex. It follows, therefore, that for each corneal facet to form the image of an object at or beyond the apex of its pyramid the object must be so far away or so small that the rays of light from it are approximately parallel. And it may be the case that images of distant objects are formed by the few facets in the direct line of sight. We know that many animals with compound eyes have keen far sight. But it is probable that each corneal facet receives rays of light from, and forms an image of, a very limited portion of an object near the eye. It is true that each such image is an inverted one, and that the image of the whole object is a mosaic of inverted fragments, but each fragment is extremely small, so that the image is not much

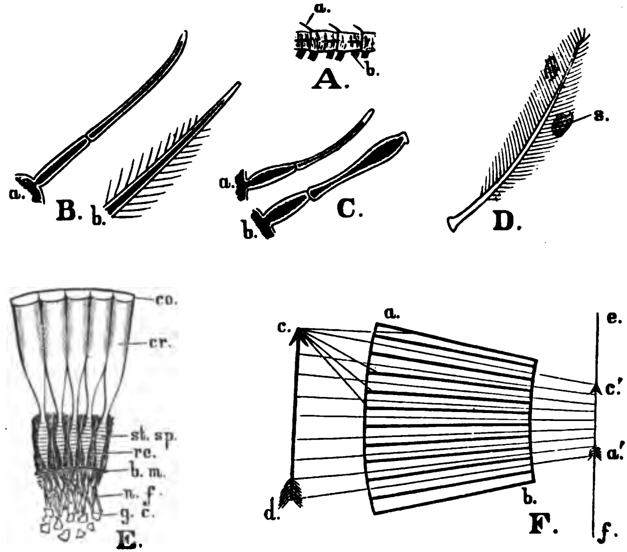

FIG. 172.—Sense organs of the crayfish. A, tactile setæ (*a*), and olfactory setæ (*b*), on the exopodite of the antennule; B, tactile setæ; C olfactory setæ; D, auditory seta with grains of sand (*s*); E, portion of section of eye; *co*, cornea; *cr*, crystalline cone; *st.sp*, striated spindle; *re*, pigment; *b.m*, basilar membrane; *n.f*, nerve fibres; *g.c*, ganglion cells; F, diagram illustrating vision with a compound eye. (Morgan.)

distorted. Swiftly moving, far-seeing animals with compound eyes are also supplied with simple eyes for near objects.

19. **The Auditory Organ.** This is in the basal joint of the antennule, the opening on its dorsal surface being guarded with thickly set plumose hairs. Cut along the angles, right and left, of the dorsal surface of this joint and remove it. With this surface will come away the **Auditory Sac,** which is a transparent chitinous bag continuous with the cuticular covering of the body, being in fact a simple pouch of it. Grains of sand will be found in the sac. Syringe these out. Cut round the lips of the sac and so free it from the antennule. Slit it up, spread out its inner surface, mount in water, and examine with a high power. Note—

(*a*) The **Auditory Setæ,** which are almost simple hairs on the inner side of the sac arranged in parallel lines. Each hair is seated on a bulbous enlargement, into which enters—

(*b*) A branch of the **Auditory Nerve.** This nerve divides up on the outer surface of the sac, and sends a branch into each sensitive seta.

Sound-waves produced in the water by concussion pass into the auditory sac, and, setting in motion the grains of sand amongst the setæ, produce the sensation of sound.

20. **Olfactory Setæ** are found on the ventral side of some joints of the exopodite of the antennule. Each seta is two-jointed, the outer joint being flattened and swollen towards the end.

21. **Tactile Setæ** are found on various portions of the body.

Reproduction.

22. The mother-cells of the non-motile **Spermatozoa** line the cavities of the lobules of the testis. The spermatozoa are nucleated cells with long curved processes. They pass, on liberation, into the ducts which lead to the vas deferens, where they become aggregated into cylindrical masses by a viscid secretion of the tube.

Squeeze out the contents of a vas deferens during the breeding season, and examine it with a high power.

23. The ovary is a hollow tri-lobed sac which opens into the oviducts. Papillæ (**Ovisacs**) appear on the inner surface of the ovary. The central cell of each becomes an **Ovum**, growing at the expense of the surrounding cells, which form an investment. The ovisac bursts, the ovum is set free, and passes into the oviduct, where it is coated with a tough stalked membrane, by which it becomes attached to the swimmerets and ventral surface of the abdomen of the female. In this position they may be found in great numbers in the spring, and here they pass through the first stages of their development.

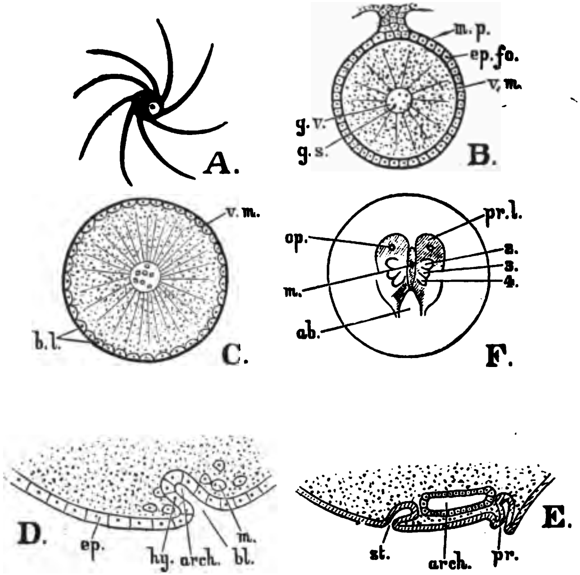

FIG. 173.—Development of the crayfish. A, spermatozoon; B, ovum; *g.v*, germinal vesicle; *g.s*, germinal spot; *ep.fo*, epithelium; C, ovum in which a blastodermic layer (*bl*) has been formed; D, invagination of the blastoderm with formation of a blastopore (*bl*); *ep*, epiblast; *m*, mesoblast; *hy*, hypoblast; *arch*, archenteron; E, formation of *st*, stomodæum, and *pr*, proctodæum; F, formation of the embryo; *op*, optic pit; *m*, mouth; *ab*, abdomen; 2, 3, 4, appendages; *pr.l*, procephalic lobe. (Morgan.)

In order to consolidate his knowledge of the anatomy of the crayfish, the student should now make a dissection to the middle line from the side, and note the relative positions of the organs as shown in Fig. 170.

CHAPTER XVII.

THE COMMON GRASS FROG (RANA TEMPORARIA).

Two species of frogs inhabit this country—the common Grass Frog (*Rana temporaria*), and the Edible Frog (*Rana esculenta*), the former very common and abundant everywhere, the latter confined to a very few localities in the eastern counties. *Rana esculenta* is the more common species on the continent, and, because it is the larger, is the more convenient for dissection; but on account of the greater ease in obtaining *Rana temporaria*, that species is described here. Most of the statements respecting the anatomy of the one will, however, apply with equal force to the other.

In their mode of life frogs are amphibious. In the spring they gather in large numbers in ponds and streams to breed. Here the female discharges her eggs, the male fertilizing them as they emerge. Later, both sexes leave the water and live on land in damp and shady places, only taking to the water for food or to escape from pursuing enemies. On the approach of winter, they bury themselves in numbers in the mud at the bottom of the water, where they pass through their period of hibernation to awake ready for breeding on the approach of warm weather.

The frog is a voracious animal feeder, the smaller land and water animals, such as slugs, worms, beetles, etc., being greedily devoured. During the summer it becomes in good condition, storing up large quantities of fat, which is consumed during the winter sleep, from which it arouses lean and hungry, the female, however, swollen out with eggs.

The eggs are uncared for by the parents, and are hatched, without their intervention, by the heat of the sun. The well-known tadpole is first produced from the egg, and this by gradual metamorphosis becomes converted into the frog. During the whole of its life, from the time of its first emergence from the egg, it is a prey to rapacious enemies. Many fish eat it greedily, the pike and eel especially finding it a tempting morsel, and not a few birds esteem it highly and are eager to make a meal of it.

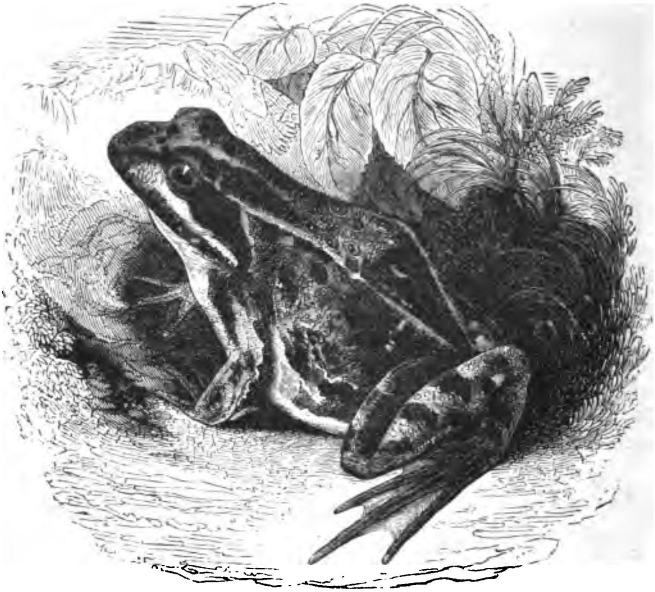

FIG. 174.—The common grass frog (*Rana temporaria*).

The tadpole breathes by means of branchiæ, but the frog, on the completion of its metamorphosis, is an air-breather by means of lungs, and immediately leaves the water to roam about on land. Nevertheless, during the whole of its life the skin functions as an organ for the absorption of oxygen and the elimination of carbon dioxide. For this purpose it is well supplied with blood-vessels, the blood in which, as in branchiæ, is only separated from the air or water in contact with it by a thin membrane, through which diffusion readily takes place.

The frog usually propels itself both on land and in water by the simultaneous extension of its very muscular hind legs. On land this results in a leap; in water, because the feet are webbed, the result is swimming. It can also float on water. It does not, as a rule, willingly completely immerse itself for any length of time, except at the period of hibernation.

The frog may be most conveniently killed by placing it in a closed space with a piece of cotton wool saturated with chloroform.

1. Have ready living and freshly killed frogs. Place others in alcohol, and one or two should be kept in a quarter per cent. solution of chromic acid for a few days before being placed in alcohol.

Study the external characteristics of the living and dead frog. Note its head and trunk, no neck being visible, and a pair of fore and hind limbs. Its skin is moist, the yellow ground colour is overlaid by much darker areas, and particularly noticeable are two large dark brown patches, one behind each eye. These are specific characters, and because of their constant presence in the temporal region of the head of this species, *Rana temporaria* has acquired its specific name. The ground colour is not constant in tint. It becomes darker if the frog be kept in a dark place, and lighter if it be exposed to light. This change is brought about by the alteration in shape of certain pigment corpuscles in the skin, and is not the effect of the direct action of light on the corpuscles themselves, but is caused by the action of light through the eye and nervous system. The temporal markings partly overlie rounded drumlike spaces with harder rims. These are the **Tympanic Membranes** which cover the cavity of the external ear.

2. The fore limbs are short, and consist of **Brachium** or **Upper Arm**, **Antibrachium** or **Fore Arm**, and **Manus** or **Hand**. The manus has four **Digits**, which are not webbed. In its natural position of rest, the antibrachium is bent inwards so that the fourth or outer digit is anterior.

The hind limbs are much longer than the fore limbs. Each consists of **Femur** or **Thigh**, **Crus** or **Shin**, and **Pes** or

Foot. The part of the pes which corresponds to the ankle in man is very much elongated, so that it forms a distinct section of the leg. It carries five long **Digits,** which are webbed, but nothing in the shape of nails are found either on them or on the digits of the fore legs. And nowhere on the skin of the frog is found any form of **Exoskeleton.** The femur in the position of rest is thrown forward, making an acute angle with the body axis, and all three parts of the hind legs are bent upon each other in the form of a capital Z. Within the limbs can be felt the appendicular portions of the **Endoskeleton.**

3. There are four openings from the exterior to the interior of the body, two median and two lateral. The anterior median opening is the wide gape of the **Mouth,** the posterior is the **Cloacal Aperture,** which is the external orifice of a vestibule, into which open the alimentary canal, the renal organs, and the sexual reproductive organs. The lateral openings are the **External Nares**; they lie on the ridges passing from the eyes to the mouth, and are the external openings of a pair of short canals which pass downward and slightly backward to the roof of the mouth. They, therefore, also lead into the digestive tract, of which they are supplementary openings. Pass a bristle through the nostrils, and note its emergence from the **Internal Nares** into the cavity of the mouth.

Open the mouth widely, and note at the back two openings—one ventral, slit-like and vertical, the **Glottis,** opening to the **Larynx** which leads to the lungs; the other above the glottis. This is irregularly shaped, and is the opening to the **Œsophagus,** or **Gullet,** leading to the stomach.

Decapitate a frog, which has been hardened in a quarter per cent. chromic acid, by a clean cut about one-eighth of an inch behind the tympanum. Study the glottis and the opening of the œsophagus, which are now very accessible. Note especially that in the frog there is no angle between the oral cavity and the larynx and œsophagus. Entirely remove the floor of the mouth, and examine anew the internal nares. Make a small hole in the tympanic membrane, and pass a bristle into the mouth cavity through the **Eustachian Tube.** Note that there are no teeth in the lower jaw, and that those in the upper jaw are distributed along the maxillary bones at the edge of the mouth and on the vomers in the roof of the mouth. Their points are turned backward, and they are not used for chewing food, but for preventing the escape of living prey.

Examine the tongue, which is attached in front, but bifurcated and free behind.

4. When in the position of rest there is a hump about the middle of the back. Anterior to this, the separate **Vertebræ** of the **Vertebral Column** may be felt beneath the skin. The hump is caused by the projection of the long **Iliac** bones, and disappears when the animal is stretched at full length.

5. In a living frog at rest the following movements may be seen :—

(*a*) The nostrils are opened and the floor of the mouth depressed whilst the mouth is filling with air. The nostrils then close, and the distension in the floor of the mouth slowly disappears, the air in the mean time being forced into the lungs through the glottis. During this time the sides of the animal are being slowly distended. The mouth acts as a pump to fill the lungs; their own inherent contractility and elasticity, as well as that of the walls of the body cavity, expel the air again when the pressure is removed. This is the operation of breathing, which goes on continuously so long as the frog is in the air.

(*b*) Slight pulsations on each side of the vertebral column above and anterior to the cloacal aperture. These are the contractions of the posterior lymph-hearts.

The Endoskeleton.

6. The **Skeleton** of the frog, with the bones connected in their natural positions, may be prepared by dissecting away as much as possible of the soft parts of the animal, then macerating the remainder in water until the rest of the soft portions readily come away on brushing with a moderately stiff brush. Hot water must not be used, or the bones will lose their con-

nections with each other. A good plan in spring is, after the viscera and so much of the muscles as possible have been removed, to suspend the frame in water with plenty of living tadpoles in it. These will clean it thoroughly in a few days. The disconnected bones should also be obtained by prolonged boiling in water.

The **Elements of the Skeleton** are of three kinds—**True Bone, Calcified Cartilage** or **Cartilaginous Bone,** and **Hyaline Cartilage.** The positions of these elements in the skeleton will be pointed out in the detailed description.

The skeleton consists of two parts—the **Axial** portion, which forms the bony case for the cerebro-spinal axis, and the **Appendicular** portion.

7. The **Axial** portion of the skeleton consists of—

i. The **Cranium.** To the cranium are attached

ii. The **Bones of the Face,** which constitute with it the **Skull.**

iii. The **Vertebral Column.**

i. The **Cranium** encloses the brain, which is in uninterrupted connection with the spinal cord through the **Foramen magnum (Occipital Foramen).**

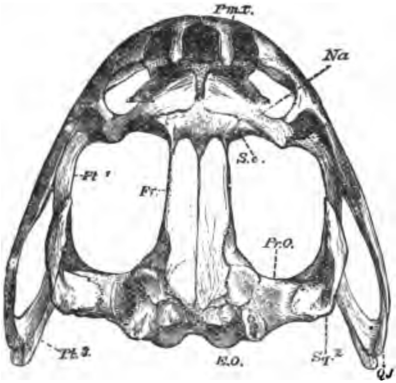

FIG. 175.—Skull of frog, seen from above. *Pmx.* premaxillary; *Na,* nasal; *S.e,* sphenethmoid; *Pt,* pterygoid; *Fr,* fronto-parietal; *Pr.O,* pro-otic; *QJ,* quadrato-jugal; *Sq*², squamosal; *E.O,* exoccipital. (Marshall.)

(*a*) Looking at the posterior aspect of the cranium, the foramen magnum is formed by two bones, the **Exoccipitals,** whose cartilaginous extremities meet in the middle line above and below the foramen. Each exoccipital has a smooth rounded process, the **Occipital Condyle,** below and outside the foramen magnum, which articulates with a corresponding depression in the atlas bone, the first bone of the vertebral column.

(*b*) The **Pro-otic** bones complete the boundary of the brain-case behind, and extend some distance up the sides. They lie one on each side of the exoccipitals. In the angle between the pro-otic and exoccipital of each side, at the upper side of the occipital condyle is the foramen, through which passes the **Vagus Nerve.** Outside this is the **Foramen Ovale,** closed by a membrane. This limits on the outer side the cavity of the internal ear, which lies in the pro-otic bone, being partially bounded, however, by the exoccipital. Trace the anterior arm of the pro-otic from the under side of the cranium. It forms but a small portion of the lateral wall of the brain-case, the remainder being wholly cartilaginous. In this portion will be seen the large foramen through which passes the **Trigeminal Nerve.**

(*c*) The floor of the cranium is formed by the **Parasphenoid,** a dagger-shaped bone with a long "blade," a short "handle," and two transverse processes which lie beneath the pro-otics. Right and left of about the middle of its anterior process are the large **Optic Foramina.** On removal of the parasphenoid it is found to underlie the plate of cartilage which closes in the brain-case at the sides.

(*d*) The roof of the cranium is formed by the **Parieto-frontal** bones. They are long bones which meet at a suture in the median line. These also overlie cartilaginous plates.

(*e*) The front of the cranium is occupied by the **Sphenethmoid,** or girdle bone. Behind, this is overlapped by the anterior edges of the parieto-frontal bones. The anterior portion of the sphenethmoid is hollowed out to form canals for the **Olfactory Nerves,** and this bone, with the nasal cartilage, encloses the **Nasal Cavities.**

ii. The **Bones of the Face.**

(*a*) In connection with the outer edge of the pro-otic bone on each side are two bones. The upper is the **Squamosal.** It is a small bone with an anterior free pointed, a posterior, and a lateral process, the latter articulated with the upper outer edge of the pro-otic.

(*b*) Underlying the squamosal, and roughly of the same shape but larger, is the **Pterygoid.** The middle lateral process of this bone is attached to the outer and under side of the pro-otic by the cartilage of the **Suspensorium,** along the under surface of which the posterior process lies. Its anterior process forms the outer side of the **Orbit,** running forward to the palatine and nasal bones. The suspensorial cartilage thus lies between the squamosal and pterygoid bones.

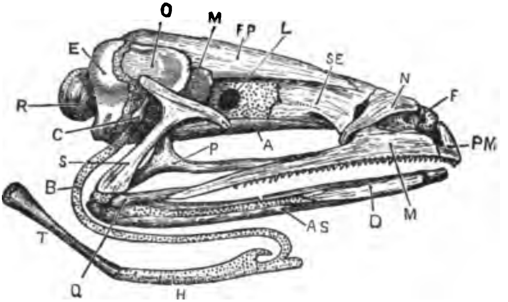

Fig. 176.—Lateral view of frog's skull. D, mandible; PM, premaxilla; M, maxilla; N, nasal; F, nostril; SE, sphenethmoid; A, parasphenoid; FP, fronto-parietal; L, optic foramen; O, pro-otic; E, exoccipital; C, columella; S, squamosal; P, pterygoid; Q, quadrato-jugal; H, hyoid; B, T, its anterior and posterior horns. (Marshall.)

(*c*) To the posterior extremity of the suspensorium is attached a small bone, the **Quadrato-jugal,** whose anterior extremity is attached to the maxilla.

The lower jaw is articulated with the posterior extremities of the suspensorium and the quadrato-jugal bone.

(*d*) The **Maxillæ** are long curved bones attached behind to the quadrato-jugals, and in front to the premaxillary bones.

(*e*) The **Premaxillary** bones close the contour of the skull in the median line in front. Each has a small ascending process which attaches it to the nasal cartilage. Both maxillary and premaxillary bones bear teeth.

(*f*) The **Nasal** bones are flat triangular plates, which lie upon the nasal cartilage. Their posterior extremities pass backwards and outwards to the maxillæ.

(*g*) The **Vomer** bones lie beneath the nasal cartilage. They are irregularly shaped, and bear teeth.

(*h*) The **Palatine** bones pass from the anterior extremity of the parasphenoid outwards to the maxillæ. Their outer extremities lie beneath the outer extremities of the nasal bones.

(*i*) The **Mandible** bounds the contour of the floor of the oral cavity. It consists of two arched compound bones, joined in the median line in front by ligament. Each arch is composed partly of bone and partly of cartilage.

(*j*) The **Hyoid** is cartilaginous in the frog. It is flat, thin, and shield-shaped, with two very long anterior, and two shorter posterior processes (cornua). It lies in the floor of the mouth, and serves as attachment for various muscles. Its anterior cornua pass backwards and upwards to their attachment to the pro-otic bones. The **Larynx** lies in the angle between the posterior cornua.

iii. The **Vertebral Column** consists of nine true **Vertebræ** and the long **Urostyle.**

As an example of one of the vertebræ, examine the fifth in detail. It consists of a thick strong **Body** on its ventral side, somewhat compressed from above downwards, and a convex **Arch** which rises laterally from it and completes the ring enclosing the spinal cord. The arch has three processes on each side, and one median process.

The **Body of the Vertebra** has a depression coated with cartilage on its anterior side, into which fits a corresponding articular head on the posterior side of the fourth vertebra. On its own posterior surface there is an articular head covered with cartilage, which articulates in a depression on the anterior surface of the body of the sixth vertebra.

The **Arch** is bony, and is not so wide from front to back as the body. In consequence of this, whilst the bodies of the vertebræ are in close contact one with another, the arches are not so. The processes of the arch are—

(*a*) A median short spine, which is pointed and projects backwards. It has an epiphysis of cartilage.

(*b*) Two pairs of lateral articular processes, which project horizontally forwards and backwards. Their extremities bear cartilaginous surfaces, on which articulate the corresponding processes of the fourth and sixth vertebræ.

(*c*) A pair of long transverse processes, which project horizontally outwards and have cartilaginous epiphyses.

The first vertebra, the **Atlas** bone, has two articular depressions in front, in which articulate the occipital condyles.

It has no transverse process. The transverse processes of the second, third, fourth, and ninth are the longest. On the transverse processes of the ninth the heads of the iliac bones articulate. The body of the eighth vertebra has no articular head on its posterior surface. It has instead a depression, into which fits a corresponding head on the anterior surface of the body of the ninth.

The **Urostyle** extends from the ninth vertebra, with which it articulates, to near the cloacal aperture. There is a sharp ridge on its dorsal side. It is hollow in its anterior portion, where its wall is perforated on each side to allow the tenth spinal nerve to emerge.

8. The **Appendicular portion of the Skeleton.**

i. The **Sternum.**

The frog has no ribs, and there is no bony or cartilaginous continuity between the bones of the fore legs and the vertebral column. The sternum, which is in the median line on the ventral side of the body, where it underlies the heart, consists of the following parts, enumerated from behind forwards:—

(*a*) The **Xiphisternum,** a thin cartilaginous plate with a deeply indented posterior border.

(*b*) The **Sternum** proper, a bony rod, broader in front than behind.

(*c*) The **Epicoracoids,** two narrow cartilages between the coracoids.

(*d*) The **Omosternum,** a bony rod broader behind than in front.

(*e*) The **Episternum,** a flat rounded plate of calcified cartilage, with a free border of hyaline cartilage.

ii. The **Shoulder-girdle.**

Passing outwards, right and left, from the epicoracoids are—

(*a*) Posteriorly, the **Coracoids.** Each is a bone with a larger flattened inner end, and a smaller rounded outer end. The outer end enters into the composition of the shoulder-joint, the inner is separated from its fellow of the opposite side by the epicoracoids.

(*b*) Anteriorly, the **Clavicles.** Each is a narrow rod of calcified cartilage, united by its inner small extremity with the epicoracoid, and by its broader outer extremity with the scapula.

(*c*) In grooves of the clavicles lie the **Precoracoids**, thin rods of cartilage, stretching from the scapula to the epicoracoids.

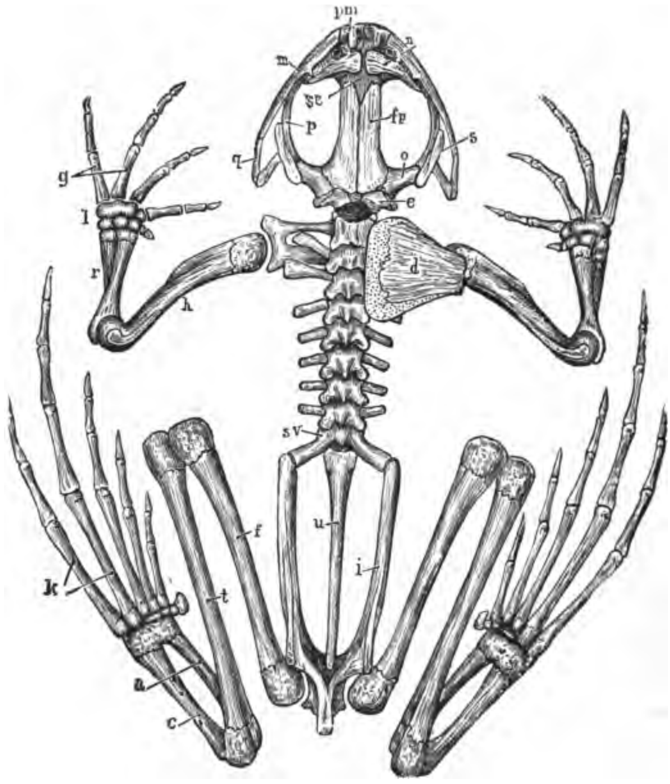

FIG. 177.—Dorsal view of frog's skeleton, with left scapula and suprascapula removed. *m*, maxilla; *n*, nasal; *se*, sphenethmoid; *p*, pterygoid; *q*, quadrato-jugal; *fp*, fronto-parietal; *s*, squamosal; *o*, pro-otic; *e*, exoccipital; *d*, suprascapula; *h*, humerus; *r*, radio-ulnar; *l*, carpal; *g*, metacarpal bones; *sv*, ninth vertebra; *u*, urostyle; *i*, ilium; *f*, femur; *t*, tibio-fibula; *a*, astragalus; *c*, calcaneum; *k*, metatarsal bones; *pm*, premaxilla. (Marshall.)

The outer extremities of the coracoids and clavicles are joined by connecting cartilage, and to this is attached on each side the **Scapula.**

(*a*) From this attachment the **Scapula** rises upward and slightly outward. It is a flat angular plate of bone, wider at the ends than in the middle. Its lower posterior angle forms with the coracoid and connecting cartilage, the **Glenoid Cavity,** in which articulates the head of the humerus.

(*b*) To the scapula is attached the **Suprascapula.** This is trapezoid in shape, of calcified cartilage, with free borders of hyaline cartilage, and is articulated with the scapula. It arches over the dorsal side of the body, the upper borders of the two bones of the opposite sides nearly meeting in the middle line over the vertebral column.

iii. **Bones of the Fore Legs.**

(*a*) The **Humerus** is the bone of the upper arm. It is roughly cylindrical, with rounded articulating extremities covered with cartilage. The upper end articulates in the glenoid cavity and forms the shoulder-joint, the lower articulates with the bone of the fore-arm and forms the elbow-joint. On the ventral surface of the bony shank is a long narrow ridge, the **Deltoid Crest,** forming the line of insertion of the deltoid muscle, which draws the limb forward. The lower portion of the humerus shows an interesting secondary sexual characteristic. In the female this part of the bone is cylindrical; in the male a **Medial Crest** arises on the dorsal side and passes around to the elbow-joint on the inner side. On this crest arises one of the muscles which flex the fore-arm.

(*b*) The bone of the fore-arm is the **Radio-ulnar,** corresponding to two bones, the radius and ulna, in man. The bone is grooved below, showing its compound character. Its upper extremity shows the cartilage-lined cavity into which is hinged the articulating process at the lower end of the humerus. Its lower extremity shows two processes, one belonging to the radius and the other to the ulna, which articulate with two of the bones of the wrist.

(*c*) The **Wrist,** or **Carpus,** consists of two rows of three bones each.

(*d*) The **Metacarpus** consists of five bones. The first, that on the side of the radius, is hidden beneath the skin. In the male it is much larger than in the female. The others are about of equal length.

(*e*) The **Phalanges.** The third and fourth fingers have each three phalanges, the first and second but two each.

iv. **Bones of the Pelvis.**

The bones of the hind legs are attached through the iliac bones to the vertebral column. The **Pelvis** is formed of three bones on each side, and each united to each in the median line. All three take part in the formation of the **Acetabulum,** the articulating cavity for the head of the femur.

(*a*) The **Ilium** is broad behind where it unites with its fellow and takes part in the formation of the acetabulum. Its anterior portion is long and narrow, and is united by its extremity to the epiphysis of the transverse process of the sacrum, or ninth vertebra.

(*b*) The **Ischium** is an irregularly shaped bone forming the posterior portion of the acetabulum.

(*c*) The **Pubis** is a triangular piece of calcified cartilage forming the ventral portion of the acetabulum.

v. **Bones of the Hind Legs.**

(*a*) The **Femur,** or **thigh** bone, is cylindrical, but slightly twisted. Its head is rounded and is capsuled into the acetabulum, from the hollow of which also passes a strong ligament to the head of the femur. Its lower extremity hinges on the shin-bone.

(*b*) The **Shin** bone (*os cruris*), like the bone of the fore-arm, shows indications of a compound structure, making it comparable to the tibia and fibula in man. It has longitudinal depressions on both surfaces. Its upper extremity is grooved to hinge with the femur, its lower extremity articulates with the bones of the tarsus.

(*c*) The **Ankle** or **Tarsal** bones are in two rows. The upper row consists of two long bones, the **Astragalus** and **Calcaneum,** with common epiphyses at either extremity, the lower of two small cartilage bones which support on the inner side of the foot what is supposed to be a rudimentary great toe.

(*d*) The **Metatarsals** are five long thin bones. They carry the phalanges at their extremities.

(*e*) The **Phalanges** of the first and second toes are two; of the third and fifth, three; and of the fourth, four.

General Internal Anatomy.

9. Pin out a frog under water, with its ventral side uppermost. One which has lain in alcohol for a few days will be preferable, as in that case the blood in the vessels will be no longer fluid. Lift up the loose skin on the ventral surface with forceps, and make a median incision from the pelvis to the mouth. Raise the flap of skin and note—

(*a*) The thin partitions which divide up the sub-epidermal space into separate **Lymph-Cavities.**

(*b*) The **Cutaneous Vessels** of the under surface of the skin. They are abundant and much branched, the branches converging towards the fore legs.

10. Sever the connection of the skin with the body-wall, and either entirely remove the ventral portion or pin it back. The following muscles of the body-wall will now come into view, and may be studied here as representatives of these muscles in the frog:—

(*a*) Two flat, thin, quadrangular **Cutaneous-pectoral** muscles, having their origin in the xiphisternum and passing forward to their attachment in the skin.

(*b*) Beneath and in front of these, passing outward from their origin in the middle line of the sternum and abdomen, a group of **Pectoral** and **Sterno-radial** muscles. They are inserted on the bones of the fore legs.

(*c*) In front of these, below the floor of the mouth cavity, the **Submaxillary (Mylo-hyoid)** muscles pass outwards right and left from the median line.

(*d*) The **Rectus Abdominis** muscles, consisting of straight parallel fibres on each side of the median line of the abdomen. They are segmented by transverse constrictions.

11. Dissect away carefully the submaxillary and the muscles which lie beneath them. This will bring into view

the hyoid cartilage. Its curved anterior horns may be seen curving backwards, and afterwards turning upwards to their attachment to the pro-otic bones.

12. Make an incision in the wall of the abdomen a little on one side of the median line from the pelvis to the sternum, taking care not to injure any organs underneath. Make a similar incision the same distance to the other side of the median line, and lift up the strip remaining. Underneath it is a large vessel, the **Anterior Abdominal Vein.** Dissect this carefully from the wall of the abdomen and remove the strip of tissue. Lift up the edge of the sternum, and note beneath it the **Heart** in its own membranous bag, the **Pericardium,** attached by folds of membrane to the upper surface of the sternum. Release this, and then continue the longitudinal cuts through the sternum with a pair of strong scissors, removing the centre piece thus set free. Stretch out widely the fore legs and pin them out. Note the organs which are now exposed in the **Pleuro-peritoneal Cavity (Cœlom,** or **Body Cavity).**

(*a*) The **Liver,** consisting of two lobes. The left is the larger and is deeply constricted.

(*b*) Behind the liver are the **Intestines,** a much-coiled tube.

(*c*) In the posterior portion of the cœlom is the **Bladder,** a large, bi-lobed, thin-walled bag. If not distended pass a blow-pipe into the cloaca and distend it with air.

(*d*) The lining membrane of the body cavity, the **Peritoneum,** is continued from the mid-dorsal region over the organs projecting into the cœlom, forming thus a thin membrane, the **Mesentery,** which suspends the viscera and through which pass the blood-vessels, nerves, and lymphatics supplied to them. Strictly speaking, the cœlom is an empty cavity, the organs usually spoken of as being in it simply bulging into it.

(*e*) The **Heart,** in its double bag, the Pericardium. The outer wall of the pericardium will have been already broken through. The space between the outer and inner walls is filled with liquid, which acts as an effective cushion for the heart.

(*f*) Beneath the left lobe of the liver is the elongated

Stomach. Pass a seeker through the œsophagus into the stomach.

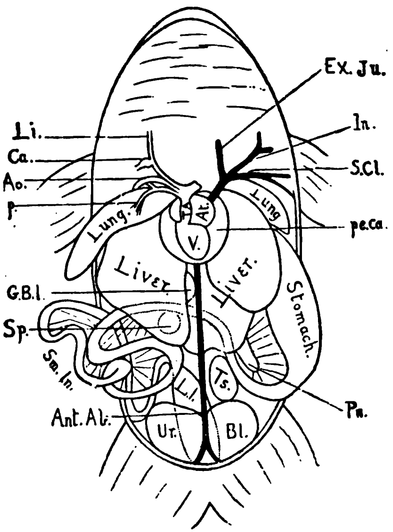

FIG. 178.—Viscera of male frog. *G.Bl*, gall-bladder; *Sp*, spleen; *Sm.In*, small intestine; *L.I*, large intestine; *Ts*, testis; *Ur.Bl*, bladder; *pe.ca*, pericardial cavity; *V*, ventricle; *At*, atrium; *Ta*, Truncus arteriosus; *Li*, lingual artery; *Ca*, carotid artery; *Ao*, aorta; *p*, pulmonary artery; *Ex.Ju*, external jugular vein; *In*, innominate vein; *S.Cl*, subclavian vein; *Pn*, pancreas. (Morgan.)

(*g*) The stomach is succeeded by the small intestine, the proximal portion of which is called the **Duodenum.** The small intestine passes suddenly into the large **Rectum,** and the rectum into the **Cloaca.**

(*h*) In the loop formed by the stomach and duodenum note the **Pancreas,** an elongated flat pale body of irregular form.

(*i*) Lift up the loop containing the pancreas and see the **Spleen,** a very small, rounded, dark-coloured body in the mesentery near the rectum.

(*j*) Also beneath the pancreas can now be seen in the male the **Testis,** an oblong white body, to whose anterior edge are attached the **Fat Bodies** in digitate masses. In the female, the **Ovary,** an irregularly shaped organ, and an immensely convoluted **Oviduct** occupy a similar position. In the breeding season ovary and oviduct occupy a large portion of the cœlom.

(*k*) The **Lungs** are in the anterior portion of the pleuroperitoneal cavity. If undistended they appear as small, dark-coloured, sacculated organs under the liver. Pass a blow-pipe through the glottis and distend them with air.

The Circulatory System.

13. The **Heart** lies beneath the anterior portion of the liver, to which the outer wall of the pericardium is attached. Its apex is conical, posterior, turned slightly to the right, and consists of the lighter-coloured, thick-walled **Ventricle**; its base is occupied by the **Atrium**, thin-walled and darker in colour. Passing from the right anterior edge of the ventricle obliquely across the atrium is the **Truncus Arteriosus**, which divides into two at the base of the heart, one division passing to the right, the other to the left. Lift up the apex of the heart, turn it forward, and note the **Sinus Venosus**, a thin-walled, dark-coloured, triangular sac lying on its dorsal surface.

14. The **Venous System.**

i. Without further dissection three veins, distinguishable from the arteries by their darker colour and thinner walls, will

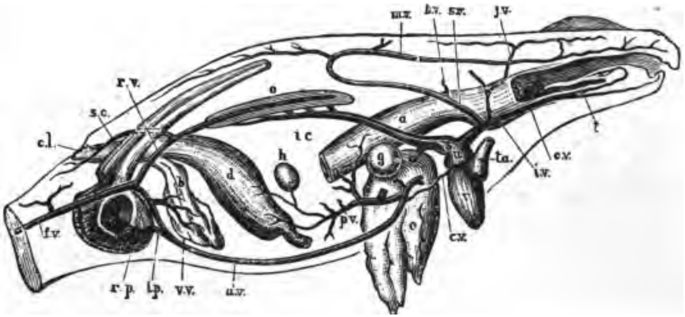

FIG. 179.—Venous system of the frog. *v*, ventricle ; *s*, sinus venosus ; *c.v*, cardiac vein ; *j.v*, internal jugular ; *s.v*, subclavian ; *m.v*, musculo-cutaneous vein ; *p.v*, hepatic portal vein : *i.c*, post-caval vein ; *a.v*, anterior abdominal vein ; *lp*, left pelvic vein ; *r.p*, right pelvic vein ; *r.v*, renal portal vein ; *s.c*, sciatic vein ; *f.v*, femoral vein. (Marshall.)

be seen on each side uniting and entering the sinus venosus at its anterior angles. They are—

(*a*) The **External Jugular,** bringing blood from the tongue and other muscles in the floor of the mouth.

(*b*) The **Innominate Vein,** formed of the **Internal Jugular** from the head and the **Subscapular** from the muscles of the shoulder-girdle.

(*c*) The **Subclavian Vein,** formed by the union of the **Cutaneous** and **Brachial Veins.** The former arises by small branches in the head, the latter in the hand and forearm.

These three veins on each side unite to form the right and left **Precaval Veins,** which open into the sinus venosus.

ii. A third large venous trunk, the **Postcaval Vein,** opens into the sinus venosus at its posterior angle.

Turn forward the apex of the heart and pin it down if necessary. The postcaval vein can now be seen. Note the entrance of the anterior abdominal vein into the liver. Dissect away carefully the portion of the liver around this vein, and note that it divides into three. One goes to each lobe of the liver, the other joins the **Hepatic Portal Vein.**

iii. Turn the posterior edge of the liver forward, and note that the hepatic portal vein entering the left lobe of the liver is made up of the following branches running through the mesentery:—

(*a*) The **Gastric Vein** from the stomach.

(*b*) The **Duodenal Vein** from the duodenum.

(*c*) The **Lieno-intestinal Vein** from the rest of the small intestine.

iv. In the anterior portion of the liver between its lobes carefully scrape away the substance, and note that a **Hepatic Vein** from each lobe joins the postcaval vein close to the sinus venosus.

v. Remove stomach, liver, and intestines, being especially careful to dissect the liver away from the postcaval vein, and place these viscera in alcohol for future examination. Open out the **Sub-vertebral Lymph-space** on one side so as to be able to lift the outer edge of the kidney, and reflect it with the ovary or testis as the case may be, over to the other side. First reflect the gonad alone, and note from the inner border of its under surface, as well as from the upper surface of the kidney, veins proceed to form a venous trunk between the kidneys. These are the **Renal** and the **Spermatic** or **Ovarian** veins, and they unite to form the postcaval vein.

vi. Lift the edge of the kidney and note a large vessel breaking up along the under surface of its outer border. This is the **Renal Portal Vein.** Turn the bladder over to the other side and trace back the renal portal vein. At the posterior extremity of the cœlom it is formed by the union of two veins—

(*a*) The **Sciatic Vein,** passing backwards at the side of the cloaca.

(*b*) The **Iliac Vein,** passing downwards and outwards in the direction of the outer side of the thigh.

The continuation backwards of the iliac vein is the **Femoral Vein,** which passes around from the outer side of the thigh. Where the femoral vein enters the cœlom it gives off a branch, the **Pelvic Vein,** which unites with the pelvic vein of the opposite side to form the anterior abdominal vein. At the root of the thigh the femoral and sciatic veins communicate by a venous trunk.

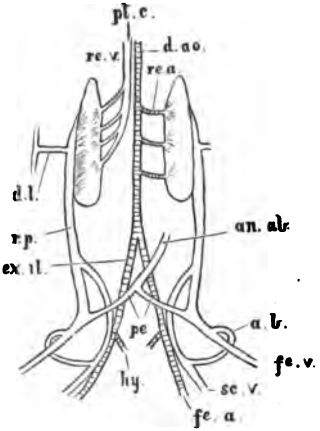

FIG. 180.—Renal portal and pelvic vessels of the frog. *a.b,* branch between the sciatic and femoral veins; *an.ab,* anterior abdominal vein; *d.ao,* dorsal aorta; *d.l,* dorso-lumbar vein; *fe.a,* femoral artery; *fe.v,* femoral vein; *hy,* hypogastric vein; *ex.il,* iliac artery; *pe,* pelvic vein; *pt.c,* postcaval vein; *re.a,* renal artery; *re.v,* renal vein; *r.p,* renal-portal vein; *sc.v,* sciatic vein. (Morgan.)

15. The **Arterial System.**

i. Return to the heart, clear away the pericardium from the roots of the great vessels connected with it on one side, cut through the factors of the precaval vein, and trace the three branches of the truncus arteriosus on that side. They are—

(*a*) The **Carotid Arch,** which is the most anterior, passes around the œsophagus, and divides at the **Carotid Gland** into two branches. One, the **Lingual Artery,** supplies branches to the floor of the mouth and tongue; the other, the **Carotid Artery,** passes upwards and supplies the brain, eyes, and other portions of the head. The carotid gland is a dilated portion of the vessel, and is of a spongy nature.

(*b*) The **Systemic Arch** is the median one. It passes upward and backward around the œsophagus to the vertebral column, where in the sub-vertebral lymph-space it meets its fellow from the other side. Before this junction it gives off branches to the vertebral column, and the head, as well as the **Subclavian Artery**, to the fore leg.

(*c*) The **Pulmo-cutaneous Arch** is the posterior arch. It divides at the level of the carotid gland into branches, the **Pulmonary Artery**, which passes to the lung on that side, and the **Cutaneous Artery**, which is distributed mainly to the skin.

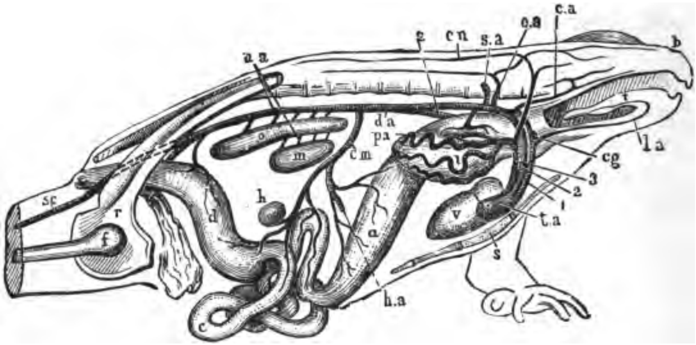

FIG. 181.—Arterial system of the frog. *v*, ventricle; *t.a*, truncus arteriosus; 1, carotid arch; 2, systemic arch; 3, pulmo-cutaneous arch; *l.a*, lingual artery; *cg*, carotid gland; *c.a*, carotid artery; *c.n*, cutaneous artery; *pa*, pulmonary artery; *s.a*, subclavian artery; *d.a*, dorsal aorta; *cm*, cœliaco-mesenteric artery; *h.a*, hepatic artery; *u.a*, urino-genital arteries; *sc*, sciatic artery. (Marshall.)

ii. The two systemic arches meet in the median line at about the level of the sixth vertebra. The right systemic arch continues along the ventral side of the vertebral column as the **Dorsal Aorta**; the left communicates with the right by a small opening, and is then continued as the **Cœliaco-mesenteric Artery**.

iii. The **Cœliaco-mesenteric** immediately divides into—

(*a*) The **Gastric Artery**, which supplies the stomach, but gives off a branch, the **Hepatic Artery**, to the liver; and

(*b*) The **Mesenteric Artery**, which supplies the intestines and spleen passing through the mesentery.

iv. The **Dorsal Aorta**, as it passes backwards in the subvertebral lymph-space, gives off several **Urino-genital Arteries** to the kidneys and gonads, branches to the muscles of the back and to the rectum, and then divides into the two **Iliac Arteries**, one of which passes into each hind leg as the **Sciatic Artery.**

16. Circulation in the Lungs.

The blood which is distributed to the lungs by the pulmonary arteries returns by the pulmonary veins. The pulmonary vein from each lung unites with its fellow above the atrium and in front of the sinus venosus, and the united veins open into the left auricle.

17. The Heart.

Remove the heart with as much of the great vessels as possible, place it in a small dissecting dish, and examine it under the dissecting microscope.

i. With a sharp knife or razor cut transversely through the **Ventricle** just below the atrium, clear from clotted blood, and examine the sections. The cavity of the ventricle appears small and slit-like, the walls very thick but spongy in texture, thicker on the left than on the right side, the inner surface of the walls covered with ridges. Two openings appear on the side attached to the atrium. The one on the right side is rounded and leads into the truncus arteriosus, the other is more central and elongated from right to left. This is the **Auriculo-ventricular Aperture** which leads into the atrium, and across it, from the dorsal to the ventral side of the atrium, can be seen the free edge of the thin membranous partition which divides the atrium into a **Right** and a **Left Auricle.** Two valves—dorsal and ventral—guard the aperture and prevent the return of blood into the auricles after it has been forced into the ventricle. Each of these is a fold of the lining membrane of the heart, attached by one edge to the wall of the aperture and hanging freely into the ventricle. Fibrous cords are attached to the edges of the valves and to the ridges on the inner wall of the ventricle, and these limit their motion so that they cannot be forced into the atrium. Two semilunar valves with free edges in the truncus arteriosus prevent blood passing back from the truncus into the ventricle.

ii. Slit up the **Left Auricle** along its outer edge, open it widely, and clear from coagulated blood if necessary. Note the entrance of the pulmonary vein on its dorsal side close to the septum.

iii. Turn the heart over on its ventral surface and open the **Sinus Venosus.** It is a triangular cavity, the two precaval veins opening into its anterior angles, the postcaval vein into its posterior angle. The opening from the sinus into the right auricle is guarded by two valves.

iv. Turn the heart back on its dorsal surface, slit up the atrial septum, and so obtain entrance into the **Right Auricle.** Note the transverse opening of the sinus venosus close to the septum.

v. Slit up the **Truncus Arteriosus** along its ventral side from the ventricle to its point of division, and lay it open. Within it is a longitudinal fold of membrane which is attached along its dorsal side in a steep spiral, but is free ventrally, and thus incompletely divides it into two chambers. Each branch of the truncus is divided internally into the three **Aortic Arches** before this division is visible from without. Cut through one of the branches, and examine the cut edge.

18. The **Course of the Circulation.**

If shortly after death by chloroform the frog be opened and the heart exposed it will be found still beating, and careful observation will show that a wave of contraction passes through the organ from sinus venosus to atrium, from atrium to ventricle, from ventricle to truncus arteriosus, the four contracting successively in the order given. The blood supplied to the right auricle by the sinus venosus, as well as that passing into the left auricle by the pulmonary vein, is forced by the contraction of the atrium into the ventricle, and cannot return on account of the closing of the auriculo-ventricular aperture by its valves. The blood in the ventricle, therefore, is of two kinds. That which has recently passed through the lung and returned to the heart by the pulmonary vein is **Arterial Blood,** newly oxygenated and pure with respect to carbon dioxide; the remainder is **Venous Blood,** containing little free oxygen and much carbon dioxide. The size and shape of the ventricle, as well as the short time the blood remains in it, prevents anything like the mixing of these two, and since the systemic blood is on the right side of the ventricle, into which side the truncus opens, this is first forced into the truncus by the contraction of the ventricle, and afterwards the purer pulmonary blood. It is prevented returning to the ventricle by the semilunar valves at the entrance of the truncus.

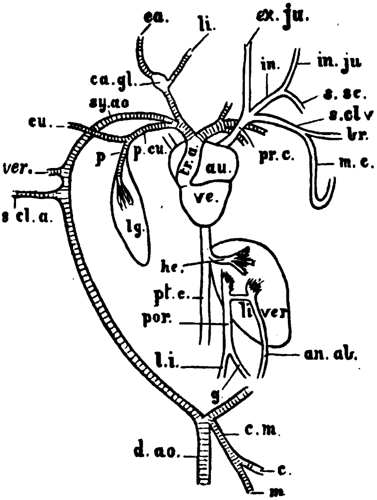

FIG. 182.—Heart and great vessels of the frog. *an.ab*, anterior abdominal vein; *au*, auricle; *br*, brachial vein; *c*, cœliac artery; *c.m*, cœliaco-mesenteric artery; *ca*, carotid artery; *ca.gl*, carotid gland; *cu*, cutaneous artery; *d.ao*, dorsal aorta; *e.x.ju*, external jugular; *g*, gastric vein; *he*, hepatic vein; *in*, innominate vein; *in.ju*, internal jugular vein; *li*, lingual artery; *l.i*, lieno-intestinal vein; *lg*, lung; *m*, mesenteric artery; *m.c*, cutaneous vein; *p*, pulmonary artery; *p.cu*, pulmo-cutaneous artery; *por*, portal vein; *pr.c*, precaval vein; *pt.c*, postcaval vein; *s.cl.a*, subclavian artery; *s.cl.v*, subclavian vein; *s.sc*, subscapular vein; *sy.ao*, systematic aorta; *tr.a*, truncus arteriosus; *ve*, ventricle; *ver*, vertebral artery. (Morgan.)

The resistance to the flow of blood is not equal in the three aortic arches. Because the course from heart again to heart is shortest by the pulmonary arteries, and because the capillaries in the lungs are comparatively large, the resistance here is least. In the carotid arches the resistance is greatly heightened by the carotid glands. The resistance in the systemic arteries is probably median. Thus when the blood is expelled by the contraction of the truncus arteriosus, the first portion, which is venous blood, is forced into the pulmocutaneous arch; the next, which is mixed blood, passes into the systemic aortas; and the arterial blood, fresh from the lungs, goes into the carotid arch, and is thus supplied to the head.

The blood returns from the anterior portion of the body by the precaval veins, having passed through but one set of capillaries; most of that supplied to the viscera of the abdomen and the hind legs passes through two sets of capillaries before returning to the heart. Thus the venous blood from the hind legs either flows by the anterior abdominal vein to the liver, whose capillaries it passes through, or by the renal portal veins to the kidneys, through whose capillaries it passes before reaching the heart by the postcaval vein. The blood returning from the stomach, intestines, pancreas, and spleen enters the liver by the hepatic portal vein and passes through its capillaries, reaching the postcaval vein by the hepatic veins.

19. The **Blood** and **Lymph.**

i. The **Blood** is a red, alkaline, coagulable and sticky liquid, consisting of a transparent fluid and almost colourless plasma, in which are suspended **Red** and **Colourless Corpuscles.** The red corpuscles are very numerous. They are oval, slightly biconvex, naked and nucleated cells, about $\frac{1}{900}$ of an inch in diameter. The nucleus is not readily seen until after treatment by dilute acetic acid, which discharges the colour, swells the cell, and brings the nucleus into view because it is not so readily acted upon by the acid as is the cytoplasm of the cell. The colouring matter is a crystalline compound called **Hæmoglobin.** The colourless corpuscles are smaller, about $\frac{1}{2500}$ of an inch in diameter and much less numerous than the red. They show amœboid movements, and many are multinuclear. They possess the power of passing through the walls of the vessels, and such migratory cells are to be found in nearly all the tissues of the body.

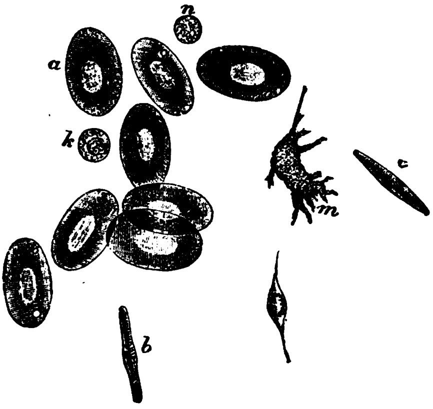

FIG. 183.—Blood-corpuscles of the frog. *a–c*, red corpuscles; *n*, *k*, colourless corpuscles at rest; *m*, active. (Ranvier.)

The circulation of the blood through the smaller vessels and capillaries can be readily seen in the web of a frog's hind foot by stretching it out on the stage of the microscope. The red corpuscles will be seen hurrying along in mid-stream, often altering their shape whilst being forced through capillaries of less diameter than themselves, but recovering it when reaching a wider place. The colourless corpuscles move along more slowly and close to the walls of the vessels.

ii. The **Lymph** is a clear transparent fluid containing corpuscles similar to the colourless corpuscles of the blood. Much of it is, in fact, derived from the blood having exuded from the capillary blood-vessels into the tissues, and thence into the lymphatic vessels and large lymph-sacs. It is returned to the blood-circulatory system by the lymph-hearts, of which there are four in the frog.

(*a*) Two **Anterior Lymph-hearts**, lying between the transverse processes of the third and fourth vertebræ. They can be observed from the ventral side of the animal after the lungs and œsophagus have been removed. Each is an oval muscular bag which receives lymph from the anterior portion of the body and by its pulsations discharges it into the vertebral vein, a branch of the internal jugular.

(*b*) Two **Posterior Lymph-hearts** are situated immediately beneath the skin, one on each side of the urostyle not far from its termination. They lie in triangular spaces among the muscles of the back, and receive lymph from the posterior portions of the body, discharging it into the communicating venous branch between the sciatic and femoral veins.

20. The **Minute Structure of the Heart and Vessels.**

i. The **Heart** is a hollow muscle. In both atrium and ventricle the outer portion consists of muscle fibres arranged circularly; interiorly, however, radial bands of fibres are found. The muscle fibres are long spindle-shaped cells which are not unfrequently branched. They show the longitudinal and transverse markings which are characteristic of the ordinary voluntary muscle fibres. The muscular bands are supported by connective tissue which passes inwards from the outer surface.

The nerve supply of the heart is derived from the pneumogastric nerve and the sympathetic system. In addition it possesses ganglia as well as smaller groups of nerve-cells.

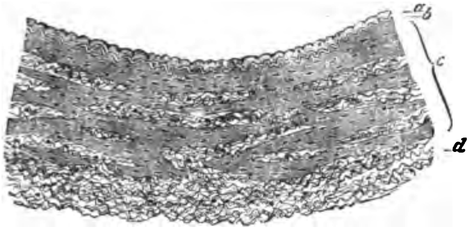

FIG. 184.—Transverse section of the wall of a vein. *a*, epithelium; *b*, elastic membrane; *c*, muscle fibres and connective tissue; *d*, connective tissue. (Quain.)

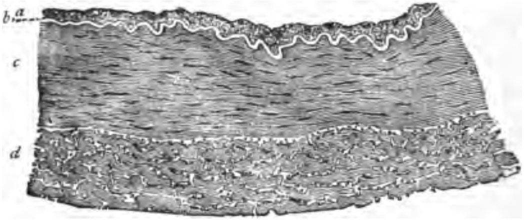

FIG. 185.—Transverse section of the wall of an artery. *a*, epithelium; *b*, elastic membrane; *c*, circularly arranged muscle fibres; *d*, connective tissue. (Quain.)

ii. **Arteries** and **Veins** are similar in their general structure. They differ chiefly in the relative amounts of muscle fibres and elastic tissue which enter into the composition of their walls. The outer wall in each is relatively thick and strong, and is composed of connective tissue, with which is mingled in the arteries much elastic tissue. The next coat is composed of unstriped muscle fibres arranged circularly. In the arteries this makes up the great part of the thickness of the wall; in the veins this layer is very thin. Within the muscle coat is a thin coat of elastic fibres, and both arteries and veins are lined by a layer of squamous epithelium. The smallest arteries and veins approach each other very nearly in structure. Many of the veins are furnished with semilunar valves, which prevent the blood from being pressed back from the heart by local pressure consequent on the contraction of muscles or the movements of limbs.

iii. The blood passes from the arteries to the veins through the **Capillaries,** the transition from the one to the other being gradual. The wall of the capillary is a single layer of nucleated endothelial cells, through which the fluid constituents of the blood, as well as many corpuscles, readily pass. The meshes of the network formed by the capillaries are frequently less in diameter than the capillaries themselves.

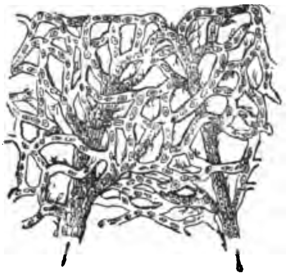

FIG. 186. — Capillary network connecting an artery and a vein in the web of a frog's foot. (Quain.)

The Respiratory System.

The **Respiratory System** consists of the **Larynx,** the **Lungs,** and their appendages. The opening from the mouth into the larynx has already been described.

21. Remove carefully the larynx and lungs, and dissect away from the former all connective tissue, etc.

i. The larynx is a wide tube of cartilage and fibrous tissue, situated between the posterior horns of the hyoid, to which, as well as to the œsophagus, it is attached. It opens posteriorly into the cavities of the lungs. It is formed of five cartilages, which are united by connective tissue, and some of which have a limited power of movement on one another. The largest cartilage is the **Cricoid.** This is of the shape of an irregular ring, and it keeps open the passage to the lungs. A pair of cartilages, convex outwards, between whose interior edges is the entrance to the larynx, are the **Arytenoid** cartilages. They are supplied with muscles, which open and close the larynx at will. Stretched between the superior and inferior angles of each are flat bands of tissue, whose anterior edges are thin and free. These are the **Vocal Cords.** The **Prearytenoid** cartilages are very small. They appear as slender rods, or are even absent or united with the arytenoid cartilages in front of which they lie.

ii. Slit down a **Lung** along its ventral surface and examine it.

The lungs are large, elliptical, thin-walled bags, narrowing at their roots, where they spring from the larynx. Their outer surfaces are smooth, and are covered by a layer of the pleuro-peritoneal membrane reflected over them from the wall of the cavity. Each is richly supplied with blood by the pulmonary artery, which spreads out over the surface of each lung, subdividing as it passes to the blunt apex. The blood is returned by the pulmonary vein, which leaves the lung at its root. The inner surface of the lung shows a coarse muscular network with wide meshes. The edges of the threads forming this network are covered with ciliated epithelial cells. Nothing but a thin structureless membrane separates the capillaries from the inner surface of the lung, and through this membrane diffuse the gases—carbon dioxide from the blood to the air in the lung, and oxygen from the air in the lung to the blood.

As has already been described, air is pumped into the lung from the mouth at each inspiration. Expiration is accomplished by the inherent elasticity of the lung and the active contractility of its abundant muscle fibres.

The blood in the pulmonary arteries differs therefore from that in the pulmonary veins in containing more carbon dioxide and less oxygen. The former is of a dark red colour, and is known as venous blood, because the blood returned to the heart by the systemic veins is of this character; whilst the latter is bright scarlet in colour, and is known as arterial blood.

It must not be forgotten that the skin of the frog is a very efficient respiratory apparatus.

The Digestive System.

The **Digestive System** consists of the **Alimentary Tract** and the **Glands** connected with it, viz. the small glands in the walls of the **Stomach** and **Intestines**, and the large glands, the **Liver** and **Pancreas.**

22. The alimentary tract consists of **Mouth, Œsophagus, Stomach,** small and large **Intestines.**

i. The **Mouth** has already been described. It is lined in great part by a layer of columnar epithelium, which is richly ciliated. In the fore part of the roof of the mouth, in front of the vomers, are the openings of the **Intermaxillary Glands.** These are small mucous glands, which secrete a sticky substance, which is deposited on the tongue when it is darted out and serves to attach to it the frog's prey.

The **Teeth** are all in the upper part of the mouth. They form a single row on the maxillary and pre-maxillary bones, and two small groups on the vomer bones. Each tooth consists of a root imbedded in the bone and a crown above the surface. It is hollow, the large cavity, containing blood-vessels and nerves, being open below. The thin wall of the tooth is mainly composed of **Dentine,** a modified kind of bone, which is covered by a layer of **Enamel,** which is the hardest substance found in the body.

ii. The **Œsophagus** is short, and is immediately above the larynx. It opens into the stomach, but no sharp line of demarcation exists between them. The **Stomach** lies above the left lobe of the liver, and is attached to the dorsal wall of the cœlom and to the liver by folds of the mesentery. The posterior portion of the œsophagus is enclosed in a lymph-sac formed by the pleuro-peritoneal membrane.

Slit open the œsophagus and stomach along the outer edge, wash it out, and note that the inner surface is thrown into longitudinal folds which extend to the **Pylorus,** a narrow constriction which separates the stomach from the duodenum.

Both œsophagus and stomach are formed of four layers of tissue. These are—

(*a*) On the outside, the peritoneum, which encloses them, forming a serous layer.

(*b*) The muscular layer, consisting of smooth muscle fibres arranged longitudinally on the outer side, circularly on the inner side.

(*c*) A sub-mucous coat of connective tissue through which pass the blood-vessels and lymphatics.

(*d*) The mucous-membrane lining. Ciliated epithelial cells are general on the inner surface of the œsophagus, and are found in the stomach. Numerous simple tubular glands exist in this membrane. They secrete the gastric juice necessary for digestion.

iii. The **Small Intestine** is suspended from the dorsal wall of the cœlom by the mesentery. It commences at the pylorus, the proximal portion parallel to the stomach being known as the **Duodenum.** Slit open the duodenum along its outer edge, wash it out, and examine its inner surface with a hand lens.

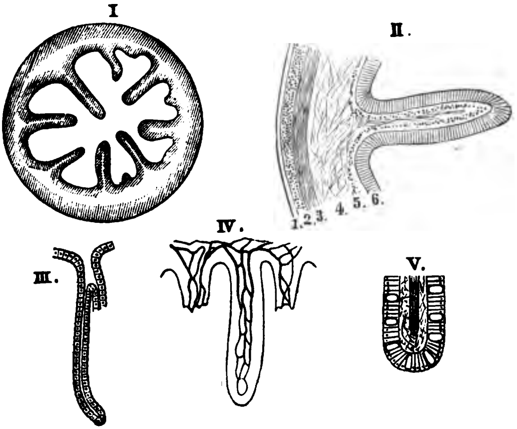

FIG. 187.—I. Transverse section of the stomach of a frog. II. A fold more highly magnified. 1, peritoneum; 2, longitudinal muscle layer; 3, concentric muscle layer; 4, submucosa; 5, muscularis mucosa; 6, mucosa containing peptic glands. III. A peptic gland of the dog. IV. Villus of the dog, showing blood-vessels. V. Villus of rabbit. (Morgan.)

The longitudinal folding of the stomach does not extend into the duodenum, but the inner surface of the latter nearest the pylorus is thrown into folds forming an irregular meshwork. Further back the folds form a double series of pouch, or waistcoat-pocket-shaped valves, whose free edges are turned backward. These prevent the return of food-substances to the stomach. Still further back the inner surface of the small intestine shows another coarse network of irregular folds which give place again to longitudinal folds extending to the large intestine or rectum. These folds largely increase the secreting and absorbing surfaces of the stomach and intestines.

The **Bile-duct,** which passes first through the pancreas and then along its outer edge, receives the secretion of the pancreas from one or more pancreatic ducts, and the common

Digestive Duct opens into the duodenum not far from the pylorus. Squeeze the gall-bladder in a freshly killed frog, and note the course of the duct by the highly coloured gall which is visible through the wall of the duct. The opening of the duct into the duodenum should be found and a bristle passed into it.

The wall of the small intestine is formed of the same four layers as have been described for the œsophagus and stomach. The mucous-membrane lining is covered by a layer of columnar epithelium, studded by large numbers of simple glands called the **Glands of Lieberkühn.** The walls are well supplied with blood-vessels, which break up into networks of capillaries beneath the mucous-membrane. The finest branches of the lymphatics, called **Lacteals** on account of the milky appearance of their contents, come into intimate relationship with the capillaries, and both closely underlie the absorbing mucous surface.

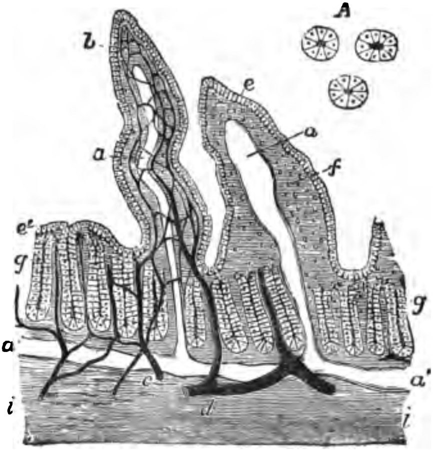

FIG. 188.—Section through the small intestine. *i*, sub-mucous layer; *c*, *d*, blood-vessels; *a*, *a'*, lacteals; *g*, mucous layer with simple glands; *b*, network of blood-capillaries in a villus; *e*, epithelium. (Quain.)

iv. The small intestine opens abruptly into the **Large Intestine.** This is in the median line. It is thin-walled, the inner surface thrown into longitudinal folds, and it appears to be non-glandular. It opens into the anterior end of the cloaca.

v. The **Cloaca** is a common vestibule into which opens the intestine, the ureters, and the ducts of the gonads. It opens to the exterior by the **Cloacal Aperture,** which is commonly called the **Anus.**

23. The **Liver** is the largest of the abdominal glands. It is held in position by the mesentery and the great blood-vessels, and is also attached to the pericardium in the manner already described.

The outer covering of the liver is a layer of the peritoneum, the mass of the organ is made up of small agglomerations, called **Lobules**, of liver-cells delimited by branches of the portal veins called **Interlobular Veins.** These send into the lobules capillaries which unite again to form an **Intralobular Vein** in each lobule. An intralobular vein is a prime factor of the hepatic vein.

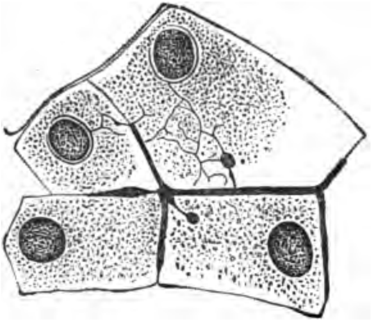

FIG. 189.—Liver-cells. (Quain.)

The supply of blood to the liver by the portal veins (anterior abdominal and hepatic portal) and the hepatic artery is most abundant. The hepatic artery breaks up into capillaries, which are mainly distributed to the external portions of the gland. The branches of the portal veins pass deeply into its substance, generally accompanied by branches of the bile-duct.

The **Liver-cells** are large, naked, polyhedral, nucleated masses of protoplasm. The prime factors of the hepatic duct are interspaces between them. In these cells the bile is secreted from the blood, and at the same time other changes take place in its composition so that the blood leaving the liver is very different from that entering it.

On the upper side of the liver, in the deep groove between its right and left lobes, is the reservoir for the bile, the **Gall-bladder.** This is a spherical transparent bag, through which can be readily seen the green **Bile** which it contains. Its walls have a coat of smooth muscle fibres. It is supplied with a duct, the **Cystic Duct,** which divides into two close to the bladder, one of the branches combining with other ducts from the liver to form the common bile-duct. This passes through the pancreas in the fold of the mesentery between the stomach and duodenum, and opens into the latter by an oblique slit in the place already described.

24. The **Pancreas** is a somewhat triangular, flat, yellow organ with in many parts an ill-defined border, lying in the gastro-duodenal portion of the mesentery. Its posterior extremity runs back to a point close to the pylorus; its anterior extremity is attached to the liver. The ducts of the pancreas open into the common bile-duct at about the middle of its length.

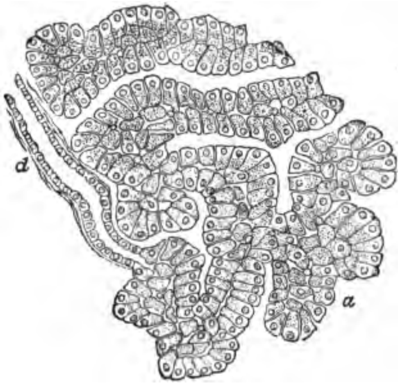

FIG. 190.—Tubules of the pancreas. *d*, commencement of a pancreatic duct. (Quain.)

The Excretory System.

The lungs and skin are excretory organs for the carbonaceous products of destructive metabolism; the kidneys excrete the nitrogenous products, which, being solids, pass in solution in water through the ureters into the cloaca.

25. The **Kidneys** are two in number, and are situated one on each side of the vertebral column in the sub-vertebral lymph-space. They are separated from the abdomen, therefore, by the peritoneum, to which their ventral surfaces, with the exception of the extreme posterior portion, are closely adherent. This membrane meets in the middle line between the kidneys and descends to form the mesentery for the small intestine. In the female the ovaries, and in the male the testes, which lie ventrally to the kidneys, are also enveloped by folds of the peritoneum, and thus hang in the cœlom by distinct mesenteries. Each kidney is about half an inch in length, flattened considerably from above downward, and with a convex outer and straight inner border.

i. Open the cloaca along both sides and find the openings of the **Ureters.** There are two of them side by side on the dorsal aspect of the cloaca, with slightly projecting lips, which form a sort of valve. Pass a bristle into one and trace the ureter up to the kidney. In the male the ureter is free in the cœlom, and along its side is the **Receptaculum Seminis,** which communicates with it by numerous small ducts; in the female the ureter is closely adherent to the oviduct. It reaches the outer edge of the kidney a short distance in front of its posterior extremity, and, passing to its dorsal surface, breaks up into tubes which penetrate its substance. These tubes—the **Tubuli Uriniferi**—follow very

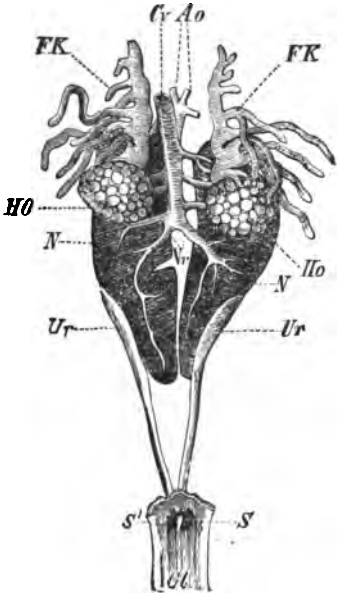

FIG. 191. — Kidneys and ovaries of the frog. *N*, *N*, kidneys; *Ur*, ureters; *Cl*, cloaca; *S'*, *S*, opening of ureters into the cloaca; *HO*, ovary; *FK*, fat masses; *Cv*, postcaval vein; *Ao*, aorta. (Wiedersheim.)

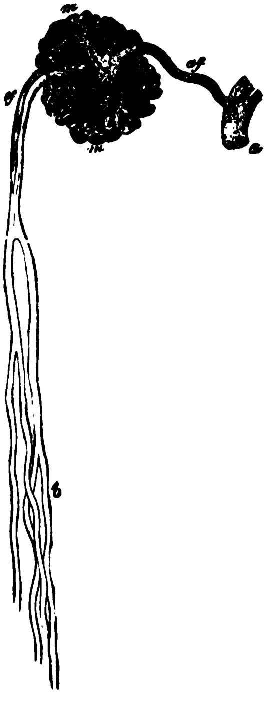

FIG. 192.—A glomerulus of the kidney. *a*, artery; *af*, afferent branch; *m*, capillaries of the glomerulus; *ef*, efferent branch. (Quain.)

devious courses in the organ, and finally terminate in small dilatations—the **Malpighian (Bowman's) Capsules.**

ii. The kidney is well supplied with blood from the **Renal Portal Vein** and the **Renal Arteries.** The former reaches the outer edge of the kidney, and breaks up on its dorsal surface. Branches pass through from the dorsal to the ventral surface, and there reunite to form the **Renal Veins.** These branches are described as being so large as scarcely to deserve the name capillaries.

The renal arteries break up just below the ventral surface of the kidney, and an ultimate branch enters each Malpighian capsule, and there forms a convoluted mass which does not quite fill the capsule. Leaving the capsule at another point, the vessel passes on to open into one of the connections between the portal and renal veins.

Thus the blood in the thin-walled vessels comes into intimate relationship with the uriniferous tubes, the arterial blood in the capsules, and the renal portal blood, through the close contact of the uriniferous tubes with the venous branches.

iii. The **Bladder,** a large, thin-walled, bi-lobed bag with a mucous membrane lining, opens into the cloaca on its ventral side. Its walls are supplied with smooth muscle fibres. It acts as a reservoir for the urine, which is continuously passing down the ureter, but is expelled from the body at irregular intervals.

The Reproductive System.

26. The **Testis** is a small, white, oval body suspended ventrally to the kidney in a fold of the peritoneum, the **Mesorchium,** which is closely adherent to its dorsal, ventral, and external sides. Through the suspending fold pass the blood-vessels and **Vasa Efferentia.** The number of these latter is not constant. They pass from the inner third of its dorsal surface to the kidney, thence to the ureter, which therefore also functions as a **Vas Deferens.**

The smallest factors of the vasa efferentia, the **Seminiferous Tubes,** may be traced back into the substance of the testis, where they terminate in irregular dilatations. These and the tubules are lined with two kinds of cells, one of which is spindle-shaped. The latter are the mother-cells of the **Spermatozoa.**

27. The **Ovaries** occupy similar positions to those of the testes. They are saccular organs, divided into chambers by septa and entirely surrounded by a fold of the peritoneum, which forms the suspensory membrane, the **Mesovarium.** Certain cells of the ovary, the **Germinal Cells,** grow to a great size at the expense of the other cells, and constitute the **Ova,** which do not pass directly into the oviduct, but are shed, when mature, into the cœlom. At the breeding season the ovaries increase enormously in size and are full of developing ova.

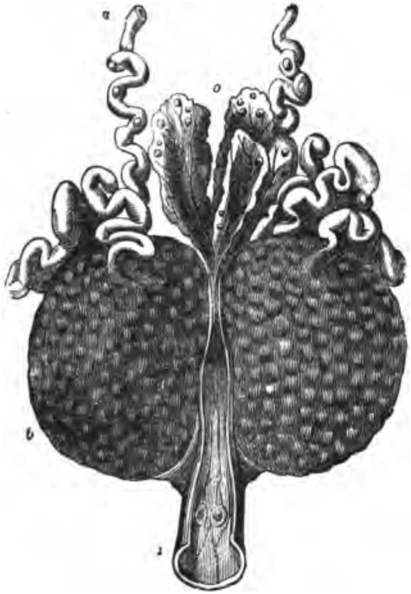

FIG. 193.—Ovaries and oviducts of the frog. *o*, ovaries; *a*, oviduct; *b*, end of the oviduct swollen with ova; *c*, cloaca. (Owen.)

The **Oviducts** are immensely convoluted tubes which are not in direct connection with the ovaries. They are suspended by a fold of peritoneum along the outer border of the kidney. Their anterior extremities open into the cœlom just behind the roots of the lungs, their posterior extremities into the cloaca just anterior to the openings of the ureters. The posterior portion of the tube is much dilated. During their passage down the oviduct, the ova become coated with the viscid secretion of its numerous simple glands.

The Nervous System.

The **Nervous System** of the frog consists of—

(*a*) A tubular central nervous **Cerebro-spinal** axis situated in its own bony cavity on the dorsal side of the animal.

(*b*) A system of paired **Nerve-trunks**, passing from the central axis and distributed to all parts of the body.

(*c*) A double chain of commissural nerve-cords with ganglia uniting many of the paired nerve-trunks on each side and united with each other. These are situated in the subvertebral lymph-space on either side of the vertebral column, and form the so-called **Sympathetic System.** It supplies nerves from its ganglia to the heart, blood-vessels, and abdominal viscera; and nerve-fibres from it accompany the branches of the spinal nerves to all parts of the body.

The cerebro-spinal axis consists of two main portions. The anterior, contained in the bony case of the skull, is the **Brain**; the posterior, enclosed in the arches of the vertebræ, is the **Spinal Cord.** There is no certain line of delimitation between the brain and spinal cord.

28. Take a frog which has lain in alcohol, pin it out under water or weak spirit with its dorsal side uppermost, and make a median longitudinal incision in the skin of the back. Reflect the skin and pin it back, or remove it, severing the septa of the dorsal lymph-sacs, and note especially the cutaneous divisions of the dorsal branches of the spinal nerves passing across the lymph-sacs to the skin.

Remove the muscles from the surface of the bony axis. Insert the point of a pair of scissors in the occipital foramen, keep it close to the surface so as not to injure the brain, cut long the outer borders of the fronto-parietals, and remove the roof of the brain-case so as to expose the brain. Turn the frog round so as to do the same for the spinal column.

The brain and spinal cord are closely invested by a very thin membrane, the **Pia Mater.** This is pigmented, especially over the spinal cord and the posterior portion of the brain. Lining the brain-case and vertebral canal is another tougher pigmented membrane, the **Dura Mater.** The space between the two is occupied by lymph. Note the chalky masses of minute calcareous particles especially along the spinal cord.

29. The **Spinal Cord** is constricted at about the middle of its length, and shows therefore an anterior and a posterior enlargement. It terminates in a sharp point, the **Filum Terminale,** contained in the urostyle. Longitudinal fissures run along its median line, both dorsally and ventrally.

30. **The Brain.** (i.) Anteriorly the spinal cord widens out into the **Medulla Oblongata (Epencephalon),** the posterior portion of the brain. It is covered by a triangular reddish plate formed mainly of blood-vessels. This is the **Choroid Plexus** of the **Fourth Ventricle.** Beneath it is the fourth ventricle, a triangular space, open now above, with tumid lateral walls and a deep longitudinal fissure in its floor.

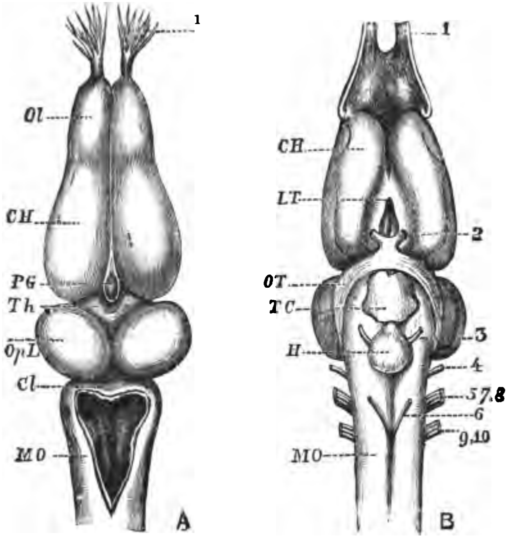

FIG. 194.—Brain of frog. A, dorsal; B, ventral aspect. 1, olfactory nerves; *Ol*, olfactory lobes; *CH*, cerebral hemispheres; *LT*, lamina terminalis; *Th*, thalamencephalon; *PG*, pineal body; *OpL*, optic lobes; *Cl*, cerebellum; *MO*, medulla oblongata; 2, optic nerves; *OT*, optic chiasma; *H*, pituitary body; 3–10, cerebral nerves. (Ecker.)

ii. The base of the triangle is in front, and is formed by the **Cerebellum (Metencephalon).**

iii. In front of the cerebellum are two rounded masses, the **Optic Lobes (Mesencephalon)**, in contact behind, diverging in front.

iv. Next is a lozenge-shaped body, the **Thalamencephalon.** Covering its anterior angle is the choroid plexus of the **Third Ventricle.** This, especially in alcohol specimens, frequently comes away with the bony roof of the brain-case attached to the dura mater. If still attached, remove it carefully, and note that it brings away with it a plug of tissue, whose outer extremity is very vascular, and inner extremity is mulberry-shaped. This plugs a small hole leading into the third ventricle. It is the pedicel of the **Pineal Body,** a non-nervous structure, which is rudimentary in the frog.

v. In front of the thalamencephalon are the two **Cerebral Hemispheres (Prosencephalon)**, elongated oval bodies, slightly separated behind, but meeting further forward with a deep fissure between them. Their anterior extremities pass directly into the elongated, rounded, **Olfactory Lobes.**

Cut through the olfactory nerves, lift the lobes, and carefully reflect the brain, cutting through the cranial nerves as they come into view. Examine the ventral surface and note from before backwards—

i. There is a small median longitudinal fissure on the cerebrum, which is continued between the olfactory lobes. The course of each olfactory nerve can be traced on the outer side of the lobe to its origin in a small bulb on the cerebrum.

ii. The cerebral hemispheres diverge behind, and the angle is occupied by a flattish plate, the **Lamina Terminalis.**

iii. Behind this is the **Chiasma of the Optic Tracts,** which come sweeping forward and downward from the optic lobes to meet in the middle line. The optic nerves pass out on either side from the optic chiasma.

iv. The **Tuber Cinereum** is a bi-lobed structure, with a thin posterior edge. Attached to it by an **Infundibulum** is the **Pituitary Body,** an oval reddish flattened body, which is not of a nervous character. It is not improbable that the pituitary body may be separated from the brain when the latter is removed in the manner here directed. In that case it will be found in a white chalky mass in the floor of the brain-case.

v. The **Crura Cerebri** are two columns of matter underneath the optic lobes and above the tuber cinereum and pituitary body.

vi. The ventral surface of the medulla oblongata is grooved longitudinally in the median line. A small papilla interrupts the groove above the pituitary body, and marks the posterior limit of the crura cerebri.

31. **Internal Structure of the Brain.** Make a median horizontal section of a hardened brain by holding it between the finger and thumb and cutting with a razor. Note the following:—

i. The cavity in the medulla oblongata open on the dorsal side is the **Fourth Ventricle.**

ii. A small canal in the median line, the **Aqueduct of Sylvius,** passes from the fourth ventricle underneath the cerebellum. It dilates between the optic lobes and communicates right and left with a cavity in each of these lobes, afterwards leading forward into the **Third Ventricle.** This is the narrow median chamber in the thalamencephalon already referred to. Its walls are formed by the **Optic Thalami.** The floor of the third ventricle is divided into an anterior portion, the lamina terminalis, and a posterior portion, the tuber cinereum, into each of which the cavity of the ventricle extends.

iii. The third ventricle leads forward into the **Foramen of Munro,** a transverse channel connecting the ventricles of the cerebral hemispheres.

iv. Each cerebral hemisphere and its connected olfactory lobe is hollow, the hollows being known as the **Lateral Ventricles.** They are connected with each other by the foramen of Munro, which is in connection with the third ventricle.

The hardened brain should be cut into a series of transverse sections and the structure studied.

32. **Internal Structure of the Spinal Cord.** The fourth ventricle of the brain leads back into the **Central Canal** of the spinal cord. This runs through the whole cord, broadening out just before its termination in the filum terminale. The ventral fissure is deep and well marked, the dorsal fissure shallow.

33. The **Cranial Nerves.** There are ten pairs of **Cranial Nerves,** which are numbered in succession from before backwards. Their origins can be studied on the ventral surface of the brain. To trace their courses, a frog should be pinned out on its side under water after a median cut has been made in the dorsal surface of the brain-case from the occipital foramen to the snout, and in that position the side-walls of the skull should be carefully removed bit by bit. The nerves of one side are—

First. The **Olfactory Nerve** arises in a small bulb on the outer surface of the cerebral hemisphere, courses forward along the olfactory lobes, and immediately passes into the nasal cavity, where it is distributed to the olfactory mucous membrane.

Second. The **Optic Nerve** arises in the optic tract. The two join and cross at the optic chiasma, after which each passes out through the optic foramen to the eyeball.

Third. The **Motor Oculi** arises near the middle line in the floor of the aqueduct of Sylvius and above the pituitary body. It is distributed to the superior, inferior, and internal rectus, and to the inferior oblique muscles of the eye on its side.

Fourth. The **Trochlear** nerve arises behind the motor oculi, and from the outer side. It supplies the superior oblique muscle of the eye.

Fifth. The **Trigeminal** nerve arises from the side of the medulla oblongata, and, in company with the sixth and seventh nerves and a branch of the sympathetic, it enters the **Gasserian Ganglion,** a large, oval ganglion, situated in the foramen, through which the nerve leaves the brain-case. The fifth is the largest of the cranial nerves, and supplies branches to the nasal cavity, the mucous membrane of the mouth, the

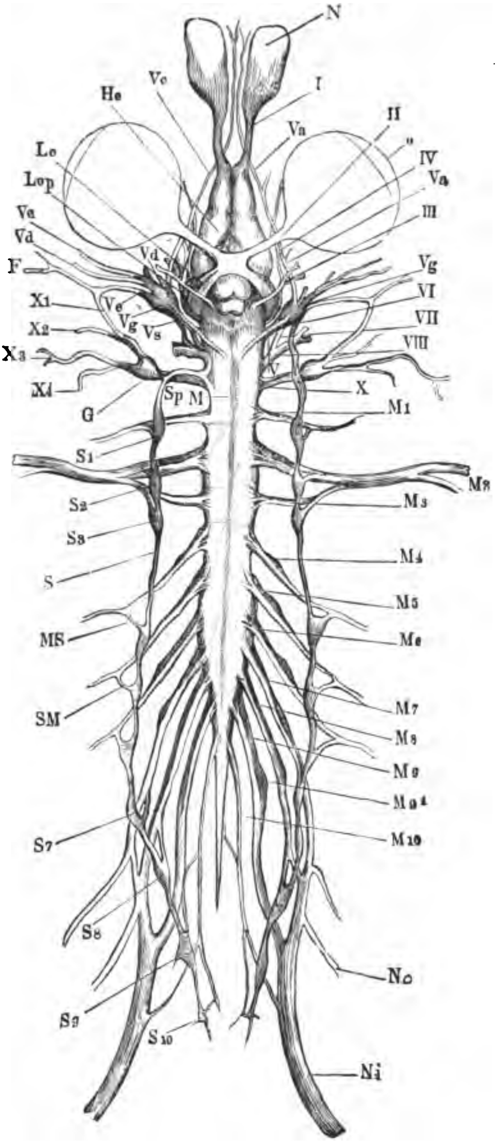

FIG. 195. — Nervous system of frog seen from beneath. I–X, cranial nerves; M1 – M10, spinal nerves; S1 – S10, sympathetic ganglia; Ni, sciatic nerve; X1, X2, branches of the glossopharyngeal; X3, X4, branches of the pneumogastric; F, facial nerve; G, ganglion of the pneumogastric; He, cerebral hemisphere; Lc, optic tract; Lop, optic lobe; M, anterior end of spinal cord; N, nasal sac; No, crural nerve; O, eyeball; S, sympathetic trunk; Vg, Gasserian ganglion. (Ecker.)

intermaxillary glands, the Harderian gland, the upper and lower jaws, and the regions round about.

Sixth. The **Abducens** nerve is small. It arises behind the fifth, and enters the Gasserian ganglion with it. It supplies the external rectus muscle of the eye, and fibres pass from it to a branch of the trigeminal.

Seventh. The **Facial** nerve arises close behind the fifth, and enters the Gasserian ganglion with it. It supplies chiefly the skin and muscles of the throat and the walls of the tympanic cavity.

Eighth. The **Auditory** nerve arises close behind the seventh. It supplies sensory nerve-fibres to the internal ear.

Ninth. The **Glossopharyngeal** nerve arises from the medulla with the tenth nerve, and leaves the cranium in company with it. It supplies a branch which unites with the facial nerve; the remainder passes to the floor of the mouth, and supplies the tongue as well as some muscles in the region of the pharynx.

Tenth. The **Pneumogastric**, or **Vagus** nerve arises in company with the ninth, with which it forms a ganglion, into which also passes a branch of the sympathetic. After leaving the ganglion it breaks up into branches which supply the skin in the region above the shoulder-girdle, and the muscles in connection with it, as well as the larynx, lungs, heart, and stomach.

34. **The Spinal Nerves.** There are ten pairs of **Spinal Nerves**, which have their origin in the cord. Each nerve arises by two roots, one dorsal, the other ventral. The dorsal root has a ganglion upon it, and the two roots join at or slightly beyond the ganglion, which lies just outside the intervertebral foramen, through which the nerve-trunk passes.

Take a frog from which the viscera of the cœlom have been removed, and the dorsal wall laid bare. Open up the sub-vertebral lymph-sinus from its anterior to its posterior limit, and the whole of the ten pairs of spinal nerves will be exposed.

First. The **Hypoglossal** nerve is small. It arises from the cord between the first and second vertebræ, and may be readily found on either side of the vertebral column, in the angle between the column and the large muscle of its side (*Levator anguli scapulæ*), which passes from the pro-otic and exoccipital bones to the supra-scapula. It supplies many muscles in and near the floor of the mouth, and passes forward to the root of the tongue, where it terminates. It will almost certainly have been met with in previous dissections near the glossopharyngeal.

Second. The **Brachial** nerve is very large. It leaves the vertebral canal between the second and third vertebræ, and forms a **Brachial Plexus** with the third spinal nerve. The brachial nerve supplies many muscles in the region of the shoulder-girdle, and passes into the arm, where it divides into two nerves, the **Ulnar** and **Radial**, which supply the fore-arm and hand.

Third. The third spinal nerve leaves the canal between the third and fourth vertebræ. It forms a plexus with the brachial nerve, and supplies certain muscles of the back and the skin.

The **fourth**, **fifth**, and **sixth** leave the canal behind the fourth, fifth, and sixth vertebræ respectively. They supply the muscles of the body-wall, and each sends a branch to the skin.

The **seventh**, **eighth**, **ninth**, and **tenth** leave the vertebral canal behind the seventh, eighth, and ninth vertebræ, and through the coccygeal canal in the urostyle, but they arise much further back. Within the canal their roots form the **Cauda equina.** They unite in the posterior portion of the sub-vertebral lymph-sinus to form the **Sciatic Plexus.** Branches leave the plexus to supply the muscles of the body-wall, the skin, the rectum, and the bladder. The tenth takes but a small part in the formation of the plexus; most of the fibres of the seventh, eighth, and ninth go to form the **Sciatic Nerve,** the largest nerve of the body. This passes into the hind leg, and divides just above the knee-joint into the **Tibial** and **Peroneal** nerves.

Dissect from the side, open the vertebral canal, and lay bare the roots of one or more of the spinal nerves of the middle region of the cord. Note the ganglion on the dorsal root, surrounded by its white **Periganglionic Gland.** Immediately beyond the ganglion a dorsal branch is given off, which supplies the muscles of the back in the region of the vertebral column, and the skin of the back. The ventral division gives off a communicating branch to the sympathetic system.

35. The **Sympathetic System.** There are ten ganglia on each side, the first closely applied to the hypoglossal nerve, the tenth, which may however be several, communicating with the tenth spinal nerve. The other ganglia communicate with the adjacent spinal nerves by branches, which, from the fourth onward, are long. Anteriorly two delicate branches pass from the first ganglion, one to the ganglion on the vagus nerve, the other to the Gasserian ganglion. In addition, small branches pass from the ganglia on the one side to those on the other.

Branches of the sympathetic are supplied to the heart, where they form a plexus on the auricle. Branches pass to the abdominal viscera, and form intricate plexuses. The largest is the **Solar Plexus,** lying in the mesentery supporting the stomach and small intestine. This is formed of branches from the third, fourth, and fifth sympathetic ganglia. Branches of the sympathetic also accompany the blood-vessels.

ORGANS OF SPECIAL SENSE.

The Ear.

There is no outer ear in the frog. The **Middle Ear,** or **Tympanum,** and the **Inner Ear,** or **Labyrinth,** alone represent the organ of hearing.

36. i. Use for preference a hardened specimen, and remove the outer skin from the dark spot on the side of the head which marks the outer limits of the ear. Immediately beneath the skin is the **Tympanic Membrane,** a thin, slightly oval membrane attached all round to a ring of cartilage. In the middle of the membrane is a white spot.

Separate the tympanic membrane from the **Annulus** and note that the white spot marks the attachment of a compound rod of bone and cartilage, bony in its middle, cartilaginous at each end. This is the **Columella Auris**, which is attached at its inner extremity to a membrane covering a hole, the **Fenestra Ovalis**, which leads into the internal ear. The **Tympanic Cavity** is somewhat flattened, and leads downwards, backwards, and inwards. It is bounded in front by the squamosal, behind by soft parts. It communicates with the back of the mouth by the **Eustachian Tube**, through which a bristle should be passed.

Cut off the head of a frog about one-eighth of an inch behind the tympanic membrane, remove the lower jaw, separate the tympanic annulus, and dissect away the soft parts around and behind the tympanum, laying this cavity completely open without displacing the columella auris from its inner attachment. It is better to work both from the outer and inner sides.

Note the columella in the roof of the cavity, separate it carefully from its inner attachment, and note the fenestra ovalis on the under side of the bony internal ear.

ii. The **Internal Ear** is tedious to dissect out, although there is no insuperable difficulty to the patient operator. It consists of a complicated sac, the **Membranous Labyrinth**, lying nearly free in a cavity of the same shape, the **Bony Labyrinth.** The membranous labyrinth is filled with a fluid, the **Endolymph**, which contains **Otoliths**, and to its walls are supplied the fibres of the auditory nerve. It is thus strictly comparable to the auditory sac of the crayfish.

The membranous labyrinth consists of the following principal parts :—

(*a*) The **Utricle**, which is the large central portion of the sac.

(*b*) From the floor of the utricle communicates, by a narrow aperture, an oval bag, the **Saccule.**

Utricle and saccule are spoken of as the **Vestibule.**

(*c*) From the utricle also open three **Semicircular Canals.** The **Anterior** lies in a vertical plane parallel to the median plane of the animal, the **Posterior** is in a vertical plane perpendicular to the median plane, the **Horizontal** is outside in a horizontal plane. Where they join the utricle they are swollen into **Ampullæ.** Two neighbouring arms of the anterior and posterior semicircular canals form a common ampulla, which is called the **Sinus Superior.**

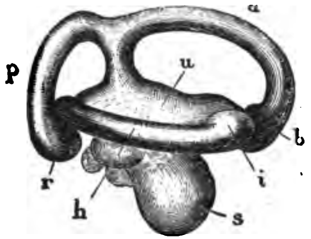

FIG. 196.—Membranous labyrinth of the right ear of the frog. *a*, *p*, *h*, anterior, posterior, and horizontal semicircular canals; *b*, *i*, *r*, ampullæ; *u*, utricle; *s*, saccule. (Marshall.)

There are also less important diverticula of the membranous labyrinth.

The fibres of the auditory nerve penetrate the walls of the utricle, saccule, and ampullæ. On the inner surface these come into close relationship with certain hair-bearing cells which are affected by the movements of the otoliths.

The **Bony Labyrinth,** formed by the pro-otic and ex-occipital bones, is of the same shape as the membranous labyrinth which is supported in it by a fluid, the **Perilymph.**

Vibrations affecting the tympanic membrane may be communicated by the columella to the membrane covering the fenestra ovalis, thence to the perilymph, and so to the endolymph. In this way air-vibrations may affect the terminations of the auditory nerves.

With a strong sharp knife cut a series of transverse sections through the head of a hardened frog, commencing just behind the posterior limit of the tympanic membrane. Study from these the bony and membranous labyrinths, the entrance to the internal ear, and as far as possible the distribution of the eighth (auditory) nerve. Portions of the membranous labyrinth should be removed and examined with a high power. The student must note, however, in order that he may not be led astray in this examination, that the periosteum lining the bony cavity is attached in places to the membranous labyrinth and comes away with it.

37. The organs of hearing of the higher vertebrata are more complicated than those of the frog. Thus, taking man as an example, there is an outer ear, consisting of a **Pinna,**

which is probably of some use in collecting air-vibrations, and a long passage, the **Auditory Canal,** at the end of which the **Tympanic Membrane** is stretched across.

In the middle ear of man, instead of the single columella of the frog, there is a chain of three bones which articulate with each other. The **Malleus,** or **Hammer Bone,** has its long handle attached to the tympanic membrane, its head articulating with the **Incus,** or **Anvil Bone,** which is on the whole parallel with the malleus. The lower extremity of the incus is attached at right angles to the **Stapes,** or **Stirrup Bone,** whose distal end is attached to the membrane of the fenestra ovalis. This latter membrane is thus set in motion when the tympanic membrane vibrates.

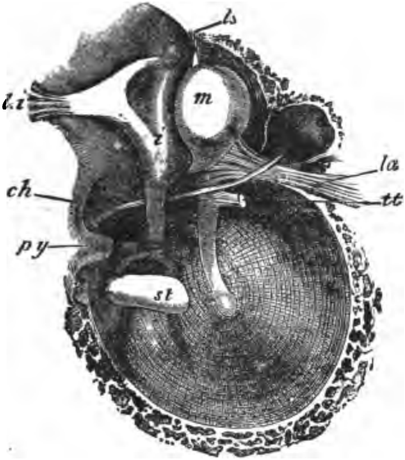

FIG. 197.—The bones of the middle ear. *m*, malleus; *i*, incus; *st*, stapes; *ch*, branch of the facial nerve.

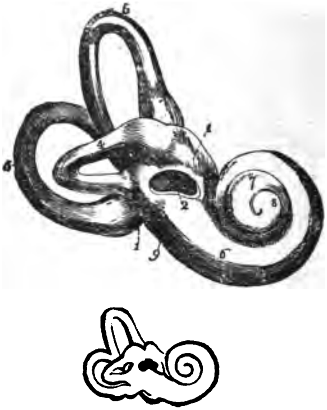

FIG. 198.—Right bony labyrinth. 1, vestibule; 2, fenestra ovalis; 3, 4, 5, semicircular canals; 6, 7, 8, the cochlea; 9, fenestra rotunda.

The vestibule and semicircular canals in man are in most respects similar to the same organs in the frog, but the saccule of man has in addition a membranous diverticulum, which is coiled about two and a half times in a helix and ends blindly at its apex. This is the **Cochlear Canal.** It is closely accompanied by two other canals, which communicate with each other at the apex of the helix, one of which, the **Scala**

Vestibuli, opens into the perilymph of the bony vestibule at the base of the helix, the other, the **Scala Tympani,** terminates on the inner side of the membrane, covering a round hole, the **Fenestra Rotunda,** on the other side of which is the tympanum. The three membranous canals lie in a helical bony canal, which is twisted round a central axis, the **Modiolus.** The whole of this portion of the organ of hearing constitutes the **Cochlea.** The partition separating the scala tympani from the scala vestibuli is a lamina of bone, the **Lamina Spiralis,** attached along its whole length to the modiolus.

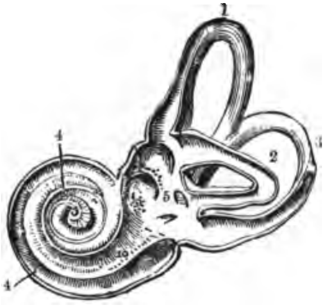

FIG. 199.—Interior of the left labyrinth. 1, 2, 3, semicircular canals; 4, cochlea; 5, vestible.

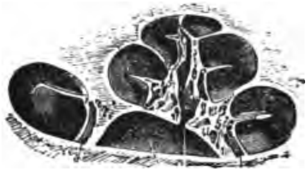

FIG. 200.—Longitudinal section from apex to base of the bony cochlea.

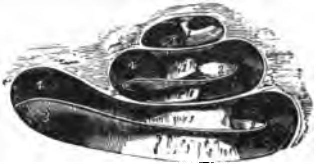

FIG. 201.—The osseous cochlea laid open. Diagrammatic.

The relationship of the three canals to each other may perhaps be better understood by likening the cochlea to a snail-shell, the helical cavity of which is divided into three along its whole length. It will be seen from the foregoing description that whilst the scalæ vestibuli and tympani must contain perilymph, the cochlear canal (or **Scala Media**) must contain endolymph.

The branch of the auditory nerve supplied to the cochlea enters the modiolus at its base, and rises up through it, giving off branches to the lamina spiralis. These have been traced into close connection with a remarkable organ lying in the membranous partition between the cochlear canal and the scala tympani. This is the **Organ,** or **Rods of Corti.**

There are about three thousand of these rods, and it is supposed that each is tuned to vibrate in unison with a sound of a certain pitch, and that by their means perception of pitch of sound or frequency of vibration is possible.

The Eye.

38. The eyes of the frog, unlike those of the crayfish, are simple. Each consists essentially of an optical apparatus for the formation of images, and a special expansion of the optic nerve with other elements for the reception of images. In addition there are highly important accessory portions.

i. The optical apparatus is a camera obscura, the **Eyeball.** This is a nearly spherical chamber, whose wall is formed posteriorly by the **Sclerotic Coat,** an extremely tough capsule of fibrous tissue and hyaline cartilage, and anteriorly by the transparent **Cornea.** The two are continuous, the cornea forming about one-fourth the surface of the eyeball, the sclerotic the remainder. Interiorly the eyeball is divided into an anterior and a posterior chamber by a pigmented diaphragm, the **Iris.** The central aperture of the iris, called the **Pupil,** is capable of being contracted by the circular muscle fibres of the iris. In higher animals the iris has also radial muscle fibres which can rapidly enlarge the pupil, but this is said not to be the case in the frog. Immediately behind the pupil is the biconvex **Crystalline Lens.** This is nearly spherical in shape, and is enclosed in a highly elastic membranous capsule, which can be tightened by special muscles, thus rendering the lens less convex. The sclerotic is lined by the **Choroid Coat,** a richly pigmented and vascular membrane which at the junction of sclerotic and cornea is continued into the iris. It is firmly attached to the sclerotic at this junction, as well as at the point of entrance of the **Optic Nerve** on the inner posterior side of the eyeball. The anterior chamber is filled with a thin watery fluid, the **Aqueous Humour,** the posterior chamber by the **Vitreous Humour,** a transparent cellular jelly enclosed in a thin membrane.

The apparatus thus described is such that rays of light from an object in front of the eye are refracted by the cornea, lens, and humours—the marginal rays being cut off by the iris—and form an inverted image on the inner surface of the back of the eyeball according to the known laws of optics.

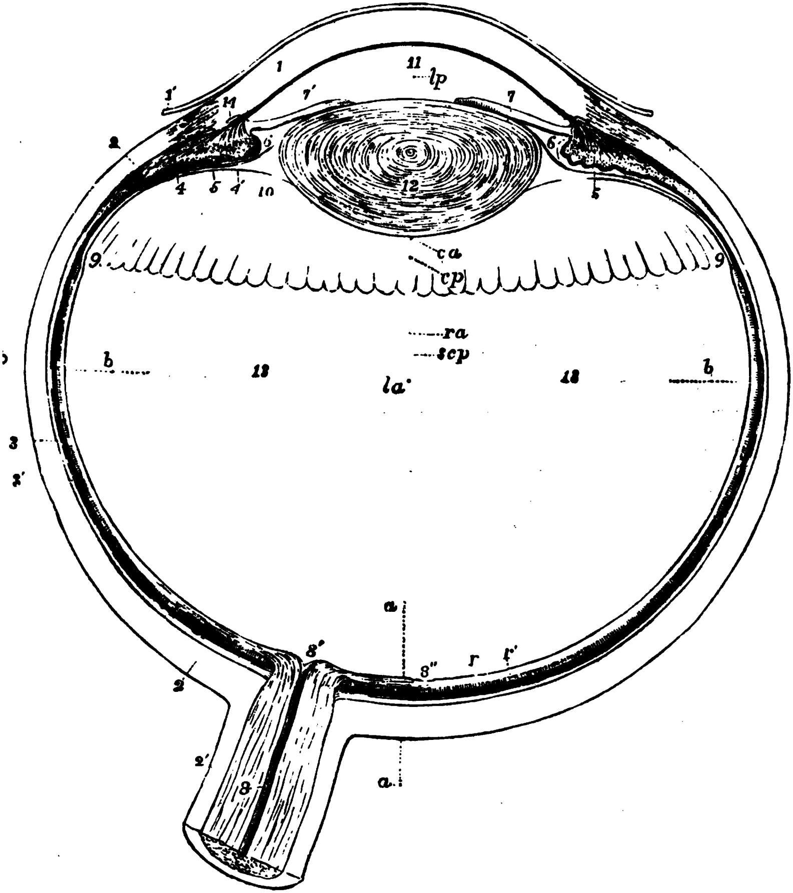

FIG. 202.—Horizontal section of the right eye. 1, cornea; 2, 2', sclerotic; 3, 3', choroid; r, r', retina; 7, 7', iris; 8, 8', optic nerve; 9, anterior limit of the retina; 11, aqueous humour; 12, crystalline lens; 13, vitreous humour.

ii. The **Receptive Apparatus** is the **Retina,** an expansion of the optic nerve, together with connective tissue and pigment elements.

The **Optic Nerve** pierces the sclerotic, enters the eyeball on its inner posterior side, and immediately spreads out between the vitreous body and the choroid coat, lining the greater portion of the posterior chamber. The **Retina** is composed of several layers. There are an **Inner** and an **Outer Limiting Membrane** connected by radially arranged **Sustentacular Cells.** These form the connective tissue support for the nervous elements of the retina. These nervous elements are, reckoning from within outwards—

(*a*) A **Layer** of **Fibres** of the optic nerve.

(*b*) The **Ganglion Layer.** This consists of nerve-cells, whose internal processes pass into the fibres of the optic nerve, and whose external processes pass into

(*c*) The **Inner Molecular Layer,** which besides these processes contains other fibrous, nervous, and connective-tissue elements.

(*d*) The **Inner Nuclear Layer** of radial nerve-fibres, with large nuclei and connective elements.

(*e*) The **Outer Molecular Layer,** similar to the inner molecular layer.

(*f*) The **Outer Nuclear Layer,** which is closely connected with the next layer.

Here is the outer limiting membrane.

(*g*) The **Layer of Rods and Cones.** The rods are about 50μ in length, with outer rounded ends. The cones are much shorter and less numerous. They have bluntly pointed outer ends. Both rods and cones show a longitudinal striation, and under certain treatment break up transversely into small discs.

(*h*) The **Pigment Layer,** which is outside the layer of rods and cones, but sends fine pigmented processes between the rods and cones as far as the external limiting membrane.

In clear sight the image of any object is formed on the retina. The rays of light which constitute the image excite the sensory terminations, rods and cones, of the optic nerve, and produce the sensation of sight.

iii. Accessory Portions.

There are two **Eyelids.** The upper has no independent power of movement. It is attached to the eyeball and moves with it. The lower eyelid—**Nictitating Membrane**—is transparent, can be raised or lowered, and serves the purpose of both eyelids of the higher animals. In dissecting, alcohol material is preferable.

The muscles of the eye may be dissected out in the following manner:—Slit the skin transversely above the eye, separate it from the eyeball and reflect it. This exposes the **Superior Rectus Muscle,** which has its origin in the fronto-parietal bone, and runs outward and forward to a broad insertion on the upper surface of the eyeball. By its contraction it turns the optic axis upwards. The wide insertion of the **Superior Oblique Muscle** is also exposed. This arises in the inner anterior corner of the orbit, and runs upwards and backwards to its insertion on the upper side of the eyeball.

Turn the animal on its back, bend back or remove the floor of the mouth, and remove the mucous membrane covering the roof of the mouth. The floor of the orbit is muscular, and a rounded projection in the roof of the mouth marks out its position. The muscular floor is the **Levator Muscle** of the eyeball. It is a broad flat muscle whose fibres run backwards and outwards, and by their contraction lift the eyeball in the orbit.

Remove the levator muscle. This will bring into view the eyeball and the following muscles attached to it:—

(*a*) The **Inferior Rectus,** which arises by a narrow tendon from the parasphenoid close to the optic foramen, and is inserted by a broad muscular portion on the lower surface of the eyeball. This muscle turns the axis of the eye downward.

(*b*) Right and left of this are the **External** and **Internal Recti,** also arising from the parasphenoid near the optic foramen, and being inserted on the outer and inner sides of the eyeball. These muscles turn the optic axis forward and backward respectively.

(*c*) In the anterior angle is the **Inferior Oblique Muscle,** arising close to the origin of the superior oblique, and being inserted on the lower side of the eyeball. The **Superior Oblique** may also be seen. These muscles rotate the eyeball about its axis.

(*d*) Remove the inferior rectus, and a portion of the **Retractor Muscle** of the eyeball is exposed. This muscle consists of three portions which surround the optic nerve, and are attached to the posterior inner surface of the eyeball. By their contraction the eyeball is drawn back and down in the orbit.

In the inner angle of the orbit is the **Harderian Gland.** This is a racemose gland which can be best exposed from the outer side of the orbit. Its watery secretion is discharged by a single duct on to the surface of the eye, which is kept moist by it. This fluid is conducted into the nasal cavity by the **Lachrymal Duct,** which opens by numerous orifices beneath the skin on the outer angle of the eye.

iv. The eyeball should now be removed for further examination. Cut through the optic nerve and attaching muscles and remove the eyeball. With scissors or sharp knife make a cut parallel to the optic axis and on the outer side, cutting off about one-third of it. The lens may now be slipped out, and the remaining structures examined in their natural positions.

Remove a portion of the retina, tease it out on a slide in weak glycerine, and examine with a high power. Many of the structural details of the retina may be made out, and particularly the rods and cones. Portions of the fresh retina should also be teased out.

The Organ of Smell.

39. The **Nasal Cavities,** lying between the external and internal nares, are surrounded by bone, and much divided up by portions of the cartilaginous skull. A general idea of their structure can be obtained by transverse sections through their region. The **Mucous Membrane** is lined by ciliated

epithelium. In the olfactory region the epithelial cells possess long processes which are in connection with the fibres of the olfactory nerve.

Organs of Taste.

40. The surface of the tongue contains stalked **Fungiform Papillæ**, whose surfaces are covered by epithelial cells with forked extremities. These are regarded as the sensory terminations of the nerve-fibres which enter the papillæ. Taste-organs are not, however, confined to the tongue, but are also found on the mucous membrane of the roof and floor of the mouth.

Organs of Touch.

41. The skin, which consists of a non-vascular **Epidermis**, covering a very vascular **Cutis**, or true skin, is well supplied with nerves. Some fibres ascend amongst the cells of the epidermis, and are no doubt sensory; but true touch areas and organs are present as tactile terminations of the sensory nerve-fibres.

The Microscopical Characters of the Tissues.

The blood and lymph have already been described.

42. The **Epithelium** is the membrane lining those cavities of the body which communicate with the exterior. Its cells are small and nucleated, and whilst they present generally a smooth surface towards the cavity they line, their inner ends are not unfrequently prolonged into one or more pointed processes. The layer of epithelium cells is single in some places, many cells thick in others. In different parts the cells have different functions. Some are, for example, absorbing cells in the intestine, secreting cells in the stomach, and sensory in the nose.

i. Gently scrape the mucous membrane in the roof of a frog's mouth, mount the scrapings in normal salt solution, and examine under a high power.

Note the nucleated epithelial cells, many with forked internal ends. The free surface is covered with cilia, which are in incessant motion if the animal has been recently killed.

ii. Scrape the inner side of your own lip, mount in saliva, examine, and note that the cells are large, flat, and nucleated (**Squamous Epithelium**).

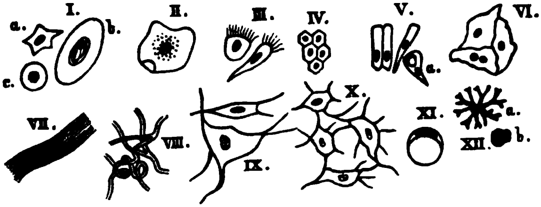

FIG. 203.—I., *a*, white corpuscle, *b*, red corpuscle of the frog; II., squamous epithelium; III., ciliated epithelium; IV., corneal epithelium; V., columnar epithelium; VI., from the peritoneum of the frog; VII., white fibrous tissue; VIII., yellow elastic tissue; IX., connective tissue corpuscles; X., corneal corpuscles; XI., fat cell; XII., pigment cells; *a*, passive; *b*, active. (Morgan.)

iii. Scrape gently the inside of the small intestine of a frog, mount in normal salt solution, and examine.

The epithelial cells are **Columnar** and nucleated, many with branched inner ends. Here and there will be seen groups of a few cells which lie side by side in their natural positions. Amongst these cells will be seen others containing or having recently discharged a drop of mucus. They are known as **Mucus, Goblet,** or **Chalice Cells,** and may be looked upon as unicellular mucous glands.

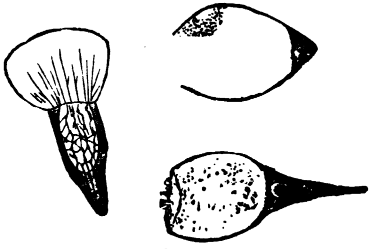

FIG. 204.—Goblet cells. (Quain.)

iv. The epithelium of the bile-ducts, the pancreas, ureters, etc., is also columnar, and in the case of the larger bile-ducts it is also ciliated. The epithelium of the bladder is formed of three layers of cells, the surface layer being polygonal in outline. The epithelium of the lungs varies in different parts. In the smallest air-spaces the epithelial cells have polygonal outlines (**Tesselated**

Epithelium), but other parts are lined with columnar ciliated cells, amongst which are goblet cells.

v. The sensory epithelium of the nose and ear has already been described.

43. **Cartilage.** Cut off a small piece from the free edge of the xiphisternum of a frog, carefully scrape from it any adhering membrane, mount in normal salt solution, and examine.

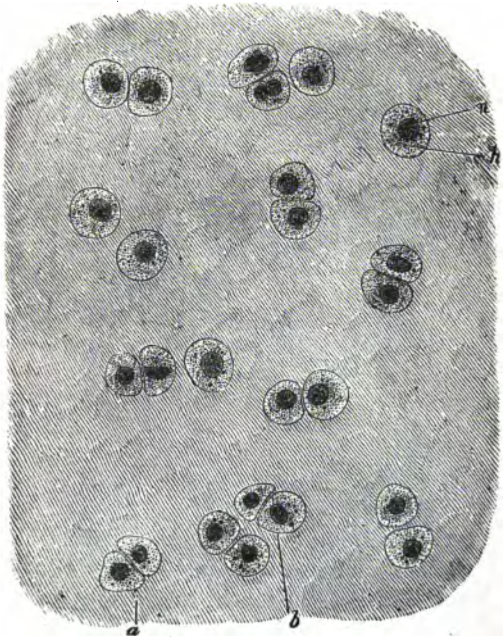

FIG 205.—Hyaline cartilage. *a*, *b*, cells; *h*, protoplasm; *n*, nucleus. (Quain.)

It consists of rounded nucleated cells lying in the lacunæ of a hyaline matrix. The cells show all the characteristics of dividing cells, and the transparent matrix is obviously an intercellular substance derived from the cells.

44. **Connective Tissue.** Remove a small piece of tendon from any of the small muscles of a frog, tease it out thoroughly, mount in normal salt solution, and examine.

It consists of very fine parallel threads of **White Fibrous Connective Tissue.**

Mount and examine in a similar manner a piece of the thin glistening connective tissue which separates the muscles of the leg from one another.

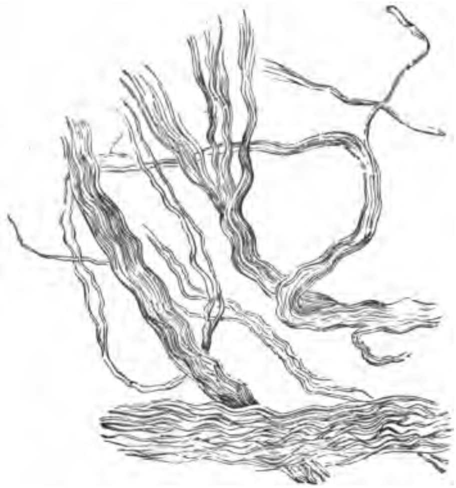

FIG. 206.—White fibrous connective tissue. (Quain.)

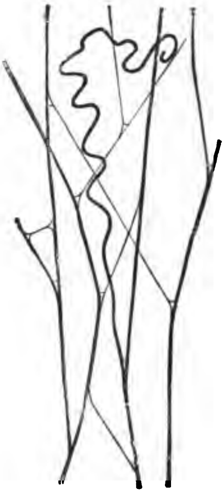

FIG. 207.—Elastic connective-tissue fibres. (Quain.)

It consists largely of the same white fibres, but amongst them will be found numbers of anastomosing **Yellow Elastic Fibres.** Run a little acetic acid under the glass, and the white fibres will swell up and lose their distinctness, whilst the yellow fibres are unaffected.

Tease out in weak glycerine a small piece of the thin edge of the tail of a tadpole which has been preserved in alcohol, or better still in a quarter per cent. solution of chromic acid, and examine.

In places, the thin layer of polygonal epidermal cells will be stripped off from the underlying tissues containing the capillaries, and here two kinds of stellate cells will be seen—larger dark-coloured pigment-cells, and much smaller nucleated, colourless, stellate cells, whose branched processes anastomose.

These latter are **Connective-tissue Corpuscles**, the formative cells of the fibres, which are intercellular like the matrix of cartilage.

45. **Bone** consists of a hardened calcified matrix containing lacunæ, in which are the formative cells—**Bone Corpuscles**—of the bone, and in addition tubes—**Haversian Canals**—which contain the blood-vessels supplying the bone.

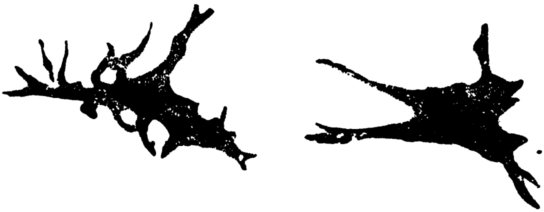

FIG. 208.—Connective-tissue corpuscles. (Quain.)

Decalcify a piece of the femur of a frog in half per cent. chromic acid, and cut transverse sections. Stain in borax carmine, and examine with a high power.

The bone is closely invested by a connective-tissue layer, the **Periosteum.** Within this is a layer of formative cells of the bone, the **Osteoblasts.** In the centre are narrow cells, also with osteoblasts. The bony matter is arranged in concentric lamellæ, marked out by circles of lacunæ, in each of which is a nucleated bone corpuscle. One lamella nearer the centre than the circumference has no lacunæ. The matrix of the bone, like that of cartilage, is an intercellular substance, produced by the living activity of the bone corpuscles, infiltrated in this case, however, with lime salts. Near the pores through which pass blood-vessels into the bone are systems of Haversian canals.

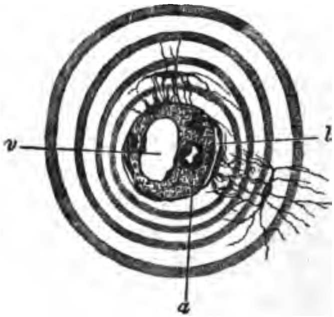

FIG. 209.—A Haversian system. *a*, artery; *v*, vein; *l*, lymphatic. (Schäfer.)

Transverse sections of the dry bone are not easy to make, and it is better to buy mounted preparations. In such a preparation of the femur of a frog the branched communicating processes — **Canaliculi**—of the lacunæ are well seen. In the living bone these are occupied by processes of the bone corpuscles.

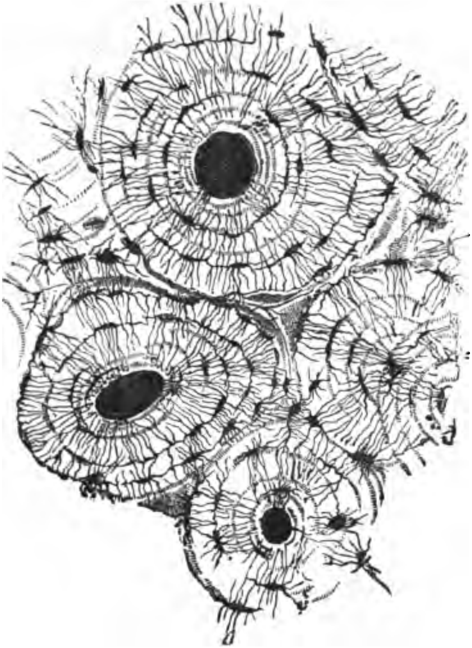

Fig. 210.—Transverse section of humerus, showing three Haversian systems. (Quain.)

The typical long bone of the higher vertebrata is made up of a number of Haversian systems, each of which consists of a canal containing the blood-vessel and a number of concentric lamellæ, separated by lacunæ with communicating canaliculi.

46. **Muscle.** i. Remove a small piece of fresh muscle from the leg of a frog, mount in normal saline solution, and examine.

The muscle is made up of transparent fibres, each of which is enclosed in a delicate sheath, the **Sarcolemma.** Where the fibre is torn or twisted the sarcolemma comes into view. The fibres are transversely striated with alternate darker and lighter area, and on this account the muscle is known as **Striped Muscle.** Run a little acetic acid under the cover-glass and the fibres become more transparent, allowing their elongated nuclei to be seen.

Tease out in glycerine, and mount a small piece of muscle which has been laid in alcohol.

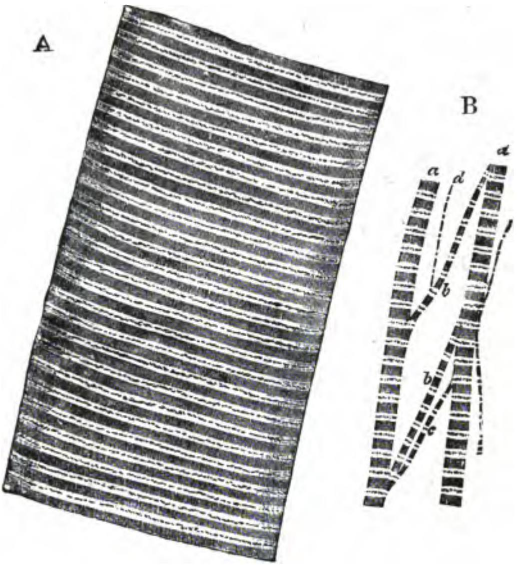

FIG. 211.—Striped muscle-fibres. B shows the breaking up of the fibre into fibrillæ. (Quain.)

The fibres split up into fine fibrils, each of which is transversely striated like the whole fibre. The striped appearance of the fibre is caused by the presence within it of a regularly arranged protoplasmic network, which is intimately concerned in the phenomena of muscular contraction.

The thin cutaneous-pectoral muscles of the frog are excellent for studying the passage of the sarcolemma of the muscle-fibres into the tendon of the muscle.

ii. Remove a small piece of the small intestine of a frog which has lain in alcohol, tease it out in weak glycerine, and examine with a high power.

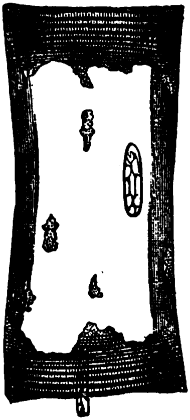

FIG. 212.—Muscle-fibre torn and showing the sarcolemma and nuclei. (Quain.)

Portions of the longitudinal and circular muscle coats will be seen, in some cases overlying each other in their natural positions, in others separated. Each coat is composed of **Unstriped Muscle** fibres—fairly long fusiform cells with oval nuclei and one or more evident nucleoli. The nuclei stain well in borax-carmine, but they show without staining.

47. **Nerve.** The nervous system is formed of nerve-fibres and nerve-cells. In a nerve-trunk the fibres are bound up together in small bundles by connective tissue, and these again into larger bundles to form

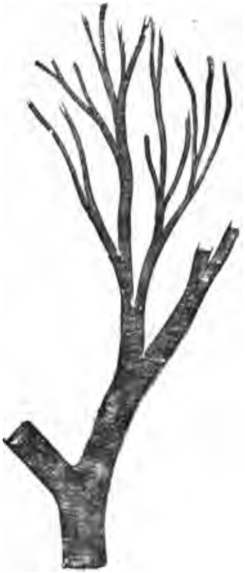

FIG. 213.—Branched muscle-fibre from the tongue of the frog. (Quain.)

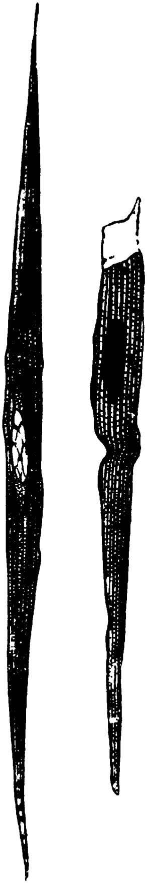

FIG. 214. — Unstriped muscle-fibres. (Quain.)

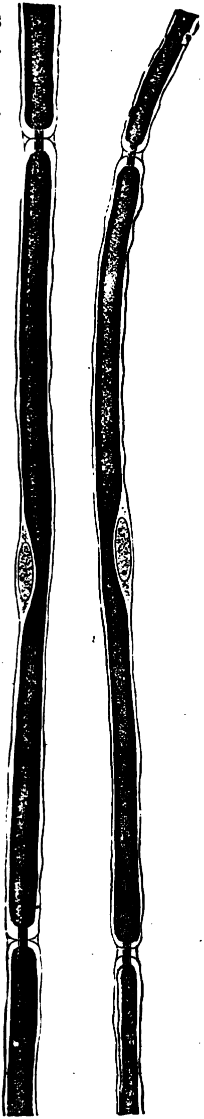

FIG. 215. — Medullated nerve-fibres. (Schäfer.)

the nerves. The connective-tissue sheaths also support nutritive blood-vessels and nerves. The spaces between the sheaths are lymph-sacs.

i. Remove a short piece of the sciatic or other large nerve of a freshly killed frog, tease out on the slide in normal salt solution, cover, and examine with a high power.

Note the fragments of the connective-tissue sheaths. The nerve-fibres are transparent cylindrical threads, with a faint indication of a central **Axis-Cylinder.** Where a fibre is bent or crushed by another crossing it, an external wall—the **Neurilemma,** or **Primitive Sheath**—is seen.

Tease up another piece of nerve in a drop of chloroform on the slide, cover, and examine.

Neurilemma and axis-cylinder are now plain. The chloroform dissolves out a fatty substance, the **Medullary Sheath,** lying between the neurilemma and the axis-cylinder.

Lay a small piece of fresh nerve for a quarter of an hour in one per cent. solution of osmic acid, tease it out in a drop of water on the slide, cover, and examine with a high power.

The medullary sheath is stained black, and its continuity is broken at intervals, dividing up the fibre into nodes and internodes. The nodes are known as the **Nodes of Ranvier.** The internodes vary in length from 10μ to 40μ. In each internode the neurilemma has at least one nucleus projecting into the medullary sheath.

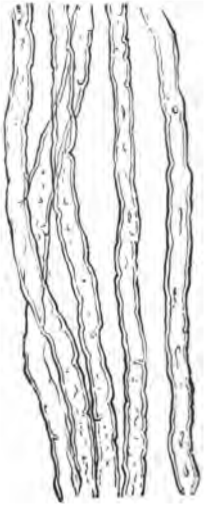

Fig. 216.—Non-medullated nerve-fibres. (Schäfer.)

The axis-cylinder is the essential part of the nerve-fibre. In transverse sections of a nerve it shows as a small dot surrounded by a circle, the neurilemma. It loses the medullary sheath near its peripheral termination, and frequently breaks up there into fine varicose fibrillæ. Non-medullated nerve-fibres are found in large numbers in the branches of the sympathetic system. They are similar in all respects to the nerve-fibres of the cerebro-spinal system, except that they have no medullary sheath.

ii. Remove a spinal ganglion from a frog, fresh or alcohol material. Place in one per cent. osmic acid for a quarter of an hour, wash, and tease out in a drop of water on a slide. Cover and examine.

Among the fibres note a large number of nerve-cells. These are of very various size, the largest reaching even to 100μ in diameter. Each has a large nucleus with nucleolus. In a few it can be seen that the cell has a single process which is continuous with a nerve-fibre; in the majority, however, the process is broken off in the teasing. They are **Unipolar Cells.** In some of the largest there is a distinct concentric fibrillation visible.

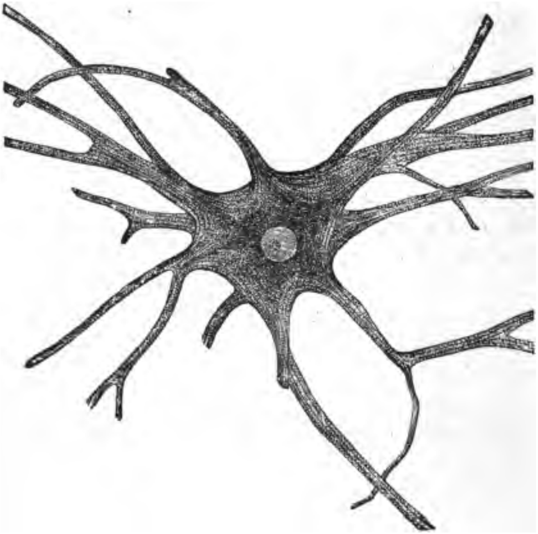

FIG. 217.—Multipolar nerve-cell. (Schäfer.)

Treat a sympathetic ganglion in the same way. Some few bipolar cells will be found—one process straight, the other twisted around it in a spiral. The straight process is continuous with a non-medullated nerve-fibre, the spiral p with a medullated fibre.

iii. Cut or procure mounted transverse sections of spinal cord, and examine first with a low, then with a h power.

Note the dorsal and ventral grooves and the central canal. Each half of the cord is composed of white matter externally and grey matter internally, the latter in transverse section appearing in the form of a crescent. In the grey matter the dorsal and ventral roots of the spinal nerves originate.

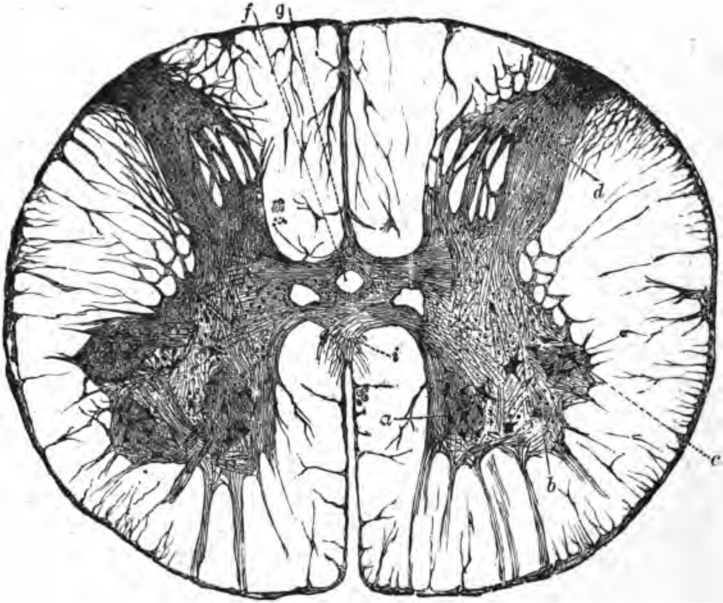

FIG. 218.—Transverse section of the spinal cord. *a, b, c,* nerve-cells in the anterior horn of grey matter; *d,* posterior horn of grey matter; *f,* central canal; *g* and *i,* commissures of grey matter. (Quain.)

The histological elements of the spinal cord are nerve-fibres, nerve-cells, and connective tissue—**Neuroglia**—the latter in comparatively large quantity supporting the nervous elements. The white matter is largely composed of medullated nerve-fibres which run longitudinally through the cord, The grey matter has large numbers of nerve-cells, most of them multipolate, although unipolate and bipolate cells are not uncommon. Large numbers of nerve-fibres pass from one side of the cord to the other by the commissure between the grooves.

Reproduction.

48. The **Seminiferous Tubes** of the **Testis** are lined by a rounded epithelium, amongst which are elongated, spindle-shaped, nucleated cells. They are the **Spermatoblasts** which give origin to the **Spermatozoa**, each of which has a short head and a long vibratile tail. The spermatozoa are set free into the seminiferous tubes, pass into the vasa efferentia, thence into the ureters, by which they pass to the cloaca and are discharged at the time of fertilization. The spermatozoa may be obtained for examination by cutting open the testis during the breeding season, and squeezing out a little of its contents.

49. The **Ovum** has a distinct cell-wall. It contains a nucleus—**Germinal Vesicle**—within which are one or several nucleoli — **Germinal Spots.** The cytoplasm of the mature ovum is coarsely granular, the granules being known as **Yolkspheres.** The ripe ova pass through the wall of the ovary into the body cavity, enter the open end of the oviduct, and so pass into the cloaca and out of the body. The **Oviduct** is a thick-walled convoluted tube, opaque and glandular through the greater portion of its length, distended and thin-walled just before its entrance into the cloaca. As the ova pass down the oviduct

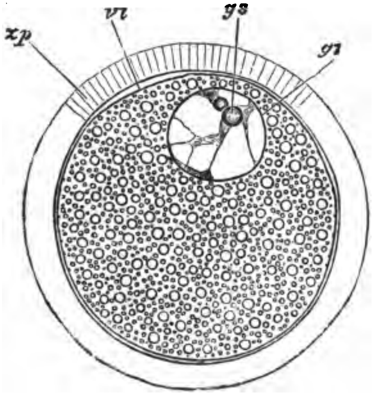

Fig. 219.—Typical ovum. *zp*, cell-wall; *vi*, inner membrane of the cell-wall; *gv*, nucleus; *gs*, nucleolus. (Quain.)

they become coated with the mucous secretion of its glands; at the moment of their expulsion the male, which tightly clasps the female around the body, pours upon them the fertilizing fluid containing the spermatozoa.

50. **Fertilization.** Before this, however, the ovum has twice expelled a portion of its nucleus (**Expulsion of Polar Bodies**), the nuclear division being of the kind called **Karyokinesis,** similar to that already described as indirect nuclear division. The portion of the nucleus remaining is the **Female Pronucleus.** A spermatozooid enters the ovum, and its substance, as a **Male Pronucleus,** fuses with the female pronucleus. Fertilization is then complete.

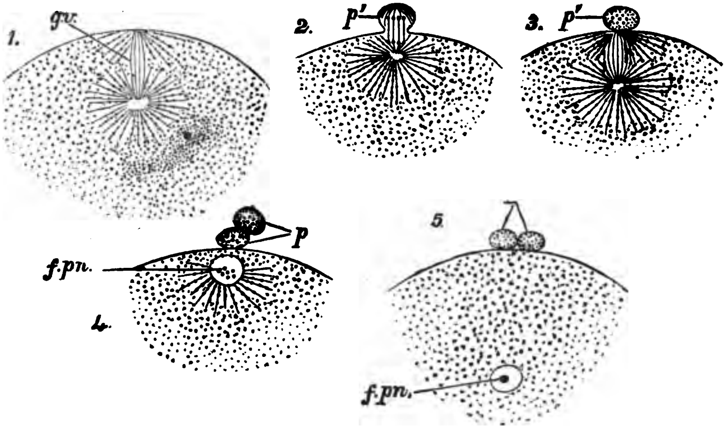

FIG. 220.—Expulsion of polar bodies from the ovum. 1, *g.v*, formation of nuclear spindle. 2, 3, *p'*, expulsion of the first polar body. 4, 5, *p*, expulsion of both polar bodies; *f.pn*, female pronucleus. (Quain.)

51. **Segmentation of the Ovum.** The ovum then commences dividing. After the first few divisions the segmentation is more rapid on the side of the nucleus, which is not quite central, than on the other side of the ovum where the food-yolk is, and in consequence the cells on that side of the ovum are soon more numerous and smaller than on this. They occupy also at this early stage less than one-half of the segmented ovum. From these smaller cells, which are darker

in colour than the others, the animal will be developed; the larger yolk-cells are subsequently absorbed by them.

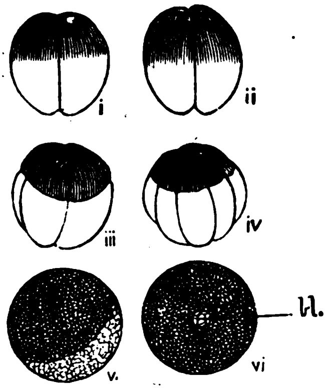

FIG. 221.—Cleavage of frog's ovum. *bl*, blastopore. (Morgan.)

The octants of the spherical ovum do not meet internally, but leave a small cavity, the **Segmentation Cavity,** which becomes larger as further divisions take place, but is finally obliterated. The pigmented cells are the **Epiblast.** They gradually advance over the surface of the sphere, increasing at the expense of the yolk-cells, until they cover it with a layer of epiblast. Before this, however, at one point on the edge of the advancing envelope, an inward and upward growth of epiblast occurs. By this means another cavity, the **Mesenteron,** or **Mid-Gut,** is formed, opening to the exterior by a small pore, the **Blastopore,** at the spot where the ingrowth commenced. The mesenteron is roofed in by a layer of cells, the **Hypoblast,** derived partly from the ingrowing epiblast and continuous with the latter at the blastopore; its floor is formed for a time by yolk-cells. Between the epiblast and hypoblast is developed a layer, several cells thick, the **Mesoblast.**

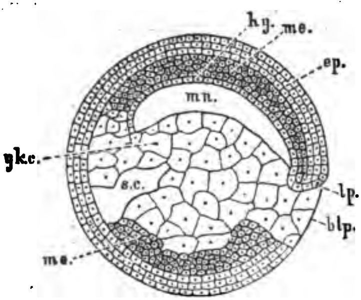

FIG. 222.—Section of frog's ovum. *blp*, blastopore; *ep*, epiblast; *me*, mesoblast; *hy*, hypoblast; *mn*, mesenteron; *yk.c*, yolk-cells; *s.c*, segmentation cavity. (Morgan.)

About this time the segmented ovum loses its spherical shape and commences elongating. The epiblast over the mesenteron by upward growth forms two longitudinal folds, the **Neural Folds,** bordering a groove, the **Neural Groove,** the floor of which is the **Neural Plate.** The folds gradually close in over the groove until they meet and fuse, forming the **Neural Canal,** which communicates at one end by a narrow tube with the blastopore.

Underneath the neural canal and parallel with it the hypoblastic roof of the mesenteron forms a rod of cells, the **Notochord,** which pierces the mesoblast and divides it into right and left halves, which eventually meet beneath the mesenteron. These are the **Mesoblastic Plates,** each of which is shortly afterwards divided into a **Vertebral Plate** next to the notochord, and a **Lateral Plate** next the mesenteron.

Before this division takes place the mesoblastic plates are cleft into a splanchnic layer on the side of the mesenteron and a somatic layer on the outer side. The cleft thus formed is the **Body Cavity,** or **Cœlom.**

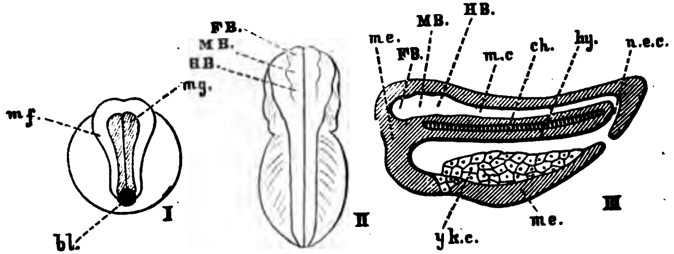

FIG. 223.—The frog embryo. I. and II., dorsal views. III., longitudinal section of an embryo in about the same stage as II. *bl*, blastopore; *ch*, notochord; FB, fore-brain; HB, hind-brain; MB, mid-brain; *hy*, hypoblast; *me*, mesoblast; *mc*, neural canal; *mf*, neural fold; *mg*, neural groove; *n.e.c*, neurenteric canal; *yk.c*, yolk-cells. (Morgan.)

52. **Development of the Nervous System.** The anterior portion of the neural canal develops three enlargements. These are the rudiments of the **Fore-brain, Mid-brain,** and **Hind-brain.** The posterior portion of the neural canal gives rise to the **Myelon,** or spinal cord.

(*a*) The primary fore-brain is the **Thalamencephalon.** The third ventricle, which it contains, is the persistent anterior vesicle of the neural canal.

(*b*) The primary mid-brain, or **Mesencephalon,** develops above into the optic lobes.

(*c*) The primary hind-brain, or **Myelencephalon,** develops into the medulla oblongata (**Epencephalon**) behind, and the cerebellum (**Metencephalon**) in front.

The nerves are developed subsequently as outgrowths of the central nervous system.

53. Early in the course of development the segmentation of the vertebral plates of mesoblast begins by the formation of transverse septa, which divide up the plates into cubical masses. A brain-case of cartilage develops around the anterior portion of the neural canal, later to be replaced in great measure by bone. Above the notochord, which extends to the posterior extremity of the body, but not further forward than the level of the mid-brain, and resting upon it, cartilaginous **Neural Arches** are developed corresponding to the septa between the mesoblastic segments, and a cartilaginous **Segmental Sheath** forms around the notochord. These segments are the **Bodies of the Vertebral Column,** in each of which a vestige of the notochord is still found in the adult animal. The cartilaginous sheath and neural arches are in great measure replaced by bone.

54. The mesenteron becomes the middle portion of the alimentary canal of the adult, and the blastopore probably persists as the anal aperture. The mouth and nares are formed by new invaginations of the epiblast, the cavities thus formed becoming continuous with the mesenteron. The margins of the mouth develop horny lips, and two suckers appear on the outer side of the embryo below the mouth.

The anterior portion of the mesenteron opens to the exterior by three **Branchial Clefts** on each side. The margins of the clefts are strengthened by cartilaginous hoops, the **Branchial Arches,** and bear three pairs of **External Branchiæ.** In the early tadpole condition water enters the mouth and escapes by the branchial clefts, thus passing over the branchiæ.

Just behind the branchial clefts on the ventral side of the digestive tract an outgrowth of the tube takes place into the cells of the mesoblast. This branches continually at its extremity, developing into the two **Lungs.**

Further back the **Liver** also arises as an outgrowth of the digestive tract, which gives rise to a mass of diverticula amongst the cells of the mesoblast. The **Pancreas** is also an outgrowth of the digestive tract.

Meanwhile the body cavity, extended upwards through the lateral mesoblastic plates, nearly meets in the middle line beneath the notochord, and so pinches the alimentary canal with its glands into the body cavity, where it is suspended by the thin mesentery, which is thus developed from the splanchnic layer of mesoblast.

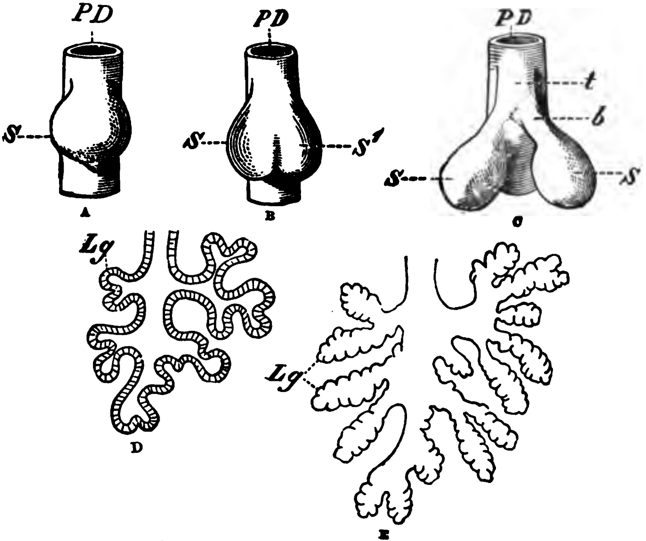

Fig. 224.—Development of the lungs from the primitive digestive tract, PD. (Wiedersheim.)

55. The **Heart** arises as a simple tube in the splanchnic layer of mesoblast beneath the pharyngeal region of the mesenteron. It is very early bent upon itself, and divided into atrium and ventricle. The atrium is shortly afterwards divided into auricles, and the sinus venosus, and truncus arteriosus are formed. In the early tadpole stage the two great arterial trunks which diverge from the truncus pass below the gill-slits and send up branches into the visceral arches which supply blood to the branchiæ. This blood is collected by efferent

branchial vessels into an **Epibranchial Artery** on each side, the two uniting further back into the **Dorsal Aorta** and passing forward as **Carotid Arteries.**

During the metamorphosis of the tadpole into the frog the following changes occur in the course of the circulation :—The first branchial arch gives rise to the carotid and lingual arteries, and so becomes the carotid arch of the adult frog. The second branchial arch loses its connection with the carotid artery, and becomes the systemic arch of the adult. The third branchial arch also loses its connection with the epibranchial, becomes the pulmo-cutaneous arch of the adult, and establishes a connection with the lungs.

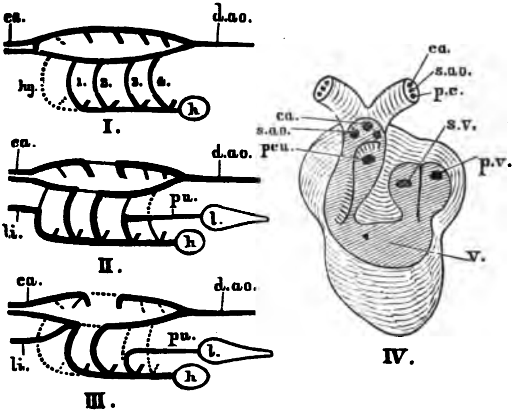

FIG. 225.—I., II., III., diagrams illustrating the development of the aortic arches in the frog. 1, 2, 3, 4, branchial arteries; *ca*, carotid artery; *d.ao*, dorsal aorta; *hy*, hyoidean artery; *h*, heart; *pu*, pulmonary artery; *l*, lung; *li*, lingual artery. IV., heart of frog dissected. *v*, ventricle; *p.v*, pulmonary vein; *s.v*, opening into sinus venosus; *p.cu*, pulmo-cutaneous opening; *s.ao*, opening of systemic aorta; *ca*, carotid opening. (Morgan.)

56. Whilst still enclosed in the egg the tadpole is furnished with two pairs of external branchiæ, increased on its escape from the egg and assumption of the free-swimming stage to three by the development of a pair of smaller posterior branchiæ. By these respiration is effected. As metamorphosis proceeds, an **Opercular Membrane** grows from the region of the **Hyoidean Arch**—anterior to the first branchial cleft—on each side, back over the external branchiæ. On the right

side and beneath, fusion of the edge of the opercular membrane with the body-wall behind the gill-slits becomes complete, on the left side an aperture is left for a time through which the water taken in at the mouth passes out. The external branchiæ then atrophy, and are succeeded in their function by **Internal Branchiæ**, developed at the edges of the branchial slits. The lungs are, however, now developing, and for a short time the animal breathes both by its lungs and its internal branchiæ. The aperture leading to the exterior finally closes, and air alone is breathed.

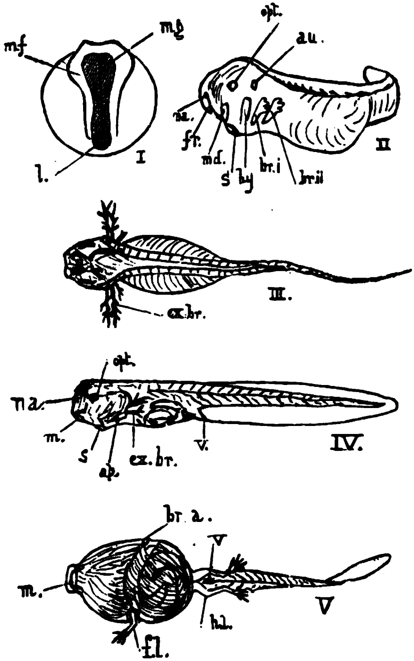

FIG. 226.—Stages in the development of the frog. I., *bl*, blastopore; *mf*, neural fold; *mg*, neural groove. II., *au*, auditory organ; *opt*, eye; *na*, nasal pit; *s*, sucker; *md*, mandibular arch; *hy*, hyoidean arch; *bri* and *brii*, 1st and 2nd branchial arches. III., *ex.br*, external branchiæ. IV., *v*, vent; *m*, mouth. V., *f.l*, fore-leg; *br.a*, branchial aperture; *h.l*, hind-leg. (Morgan.)

57. In the young embryo temporary renal organs are developed, which subsequently in great part atrophy. On each side of the body cavity in the somatic layer of mesoblast is developed a duct—the **Archinephric**, or **Segmental Duct**—which communicates behind with the cloaca, and opens to the body cavity near its anterior limit by several apertures. Just behind the apertures it forms a convoluted mass, with blind processes. This is the **Pronephros**, or **Head-Kidney**. The primitive kidney of the frog, therefore, like the kidney of anodonta, is a tube

communicating freely with the body cavity. This head-kidney atrophies after a time.

It is replaced in function by the **Wolffian Body,** or **Mesonephros,** which persists as the permanent kidney of the frog. This originates further back in the somatic mesoblast as a convoluted mass of tubules, which subsequently branch. Each communicates with the archinephric duct, and ends in a glomerulus. The posterior portion of the archinephric duct becomes therefore the **Wolffian Duct (Ureter)**.

The oviduct of the female originates as a longitudinal development from the archinephric duct, and opens far forward into the body cavity and behind into the cloaca. A somewhat similar duct is similarly developed in the male, but it is functionless and afterwards atrophies.

The vasa efferentia are developed as outgrowths of the primary tubules of the Wolffian bodies. They communicate with the testes, and thus it comes about that the ureters (Wolffian ducts) function also as vasa deferentia.

CHAPTER XVIII.

SPECIAL PHYSIOLOGY OF THE CŒLOMATA.

We have seen that of the animal types described here, each individual amœba, vorticella, and paramæcium is morphologically a single cell, although with a high degree of complexity. Into this cell are taken solid particles of food, which, consisting mainly of protoplasm, are broken down to some extent before being constructed anew and elevated into the animal matter of the living body. Destructive metabolism is constantly going on, the processes supplying heat and force to the animal with the production of carbonaceous and nitrogenous waste which is excreted from the cell.

Such unicellular animals belong to the great division of **Protozoa.** Multicellular animals which show structural differentiation are **Metazoa.** Like the other types of animals, all such have a digestive cavity into which the food enters, and in which digestion takes place. But there is this distinction between hydra on the one hand, and anodonta, astacus, and rana on the other, that whereas the former has but one cavity, the digestive cavity, the latter have each a distinct body cavity —**Cœlom**—with a distinctive vascular system containing a nutrient and oxygenated fluid, as well as a digestive cavity—**Enteron.** Hydra belongs to that division of the metazoa called **Cœlenterata,** the others to the division **Cœlomata.**

In spite of the fact that hydra possesses a digestive cavity, we have seen that its digestion is largely intracellular. In the cœlomata intracellular digestion does not prevail, but digestive juices are poured into the digestive tract from the glands which open into it, and there food-stuffs are brought into solution and prepared for absorption into the circulation.

There is no reason to suppose that the chemistry of the metabolism of the cœlomata differs, except perhaps in degree of complexity, from that of the protozoa, and the statement of profit and loss set out in the section on Amœba will certainly apply with equal force to all these animals. But their greater complexity of structure with their accompanying differentiation of organs and functions involves a physiological division of labour, and necessitates a vascular system, with its contained nutrient fluid to carry food and oxygen to distant organs, and to bring the products of destructive metabolism to the organs which excrete them.

Although the protoplasm of the cells of the higher animals is **contractile, irritable, automatic, absorptive, metabolic**, and **reproductive**, yet it may be said generally that those cells which exhibit one or the other of these properties in the highest degree are collected together into groups constituting organs where their united action can affect the whole organism. Thus the cells in which the property of contractility is most highly developed form the muscles and ciliated epithelium; the most irritable and automatic cells constitute the nervous system; those which are especially endowed with the power of reproducing the animal occur in the gonads—ovary and testis; and all living cells are necessarily absorptive and metabolic.

Within the limits of this section it is impossible to do more than touch upon the leading features of animal physiology. What follows will apply, with some reservations which will be noted, to all the types of cœlomata here presented.

Nutrition.—The food of animals must consist of organic compounds already elaborated in plants or animals, together with water and some inorganic salts, and these food-stuffs may be referred to three classes, viz.—

(*a*) Nitrogenous compounds such as proteids.

(*b*) Carbonaceous compounds such as carbohydrates, of which starch is an example, and fats.

(*c*) Water and mineral salts.

The rendering soluble and diffusible such of these substances as are insoluble is the process of digestion. The same treatment is not suitable for each of them, and therefore different parts of the digestive tract are modified to deal with the different food-stuffs. Insoluble proteids are converted into peptones, starch into sugar, and fats to some extent into soaps, all of which are diffusible through animal membranes. Mineral salts which are foods are all more or less soluble.

The digestive tract has already been described as an open channel leading through the body, commencing at the mouth and terminating at the anus, dilated in some parts, constricted in others, but at no point communicating by open tube with the blood-vascular or lymphatic systems by which the products of digestion are carried away from it. Into this channel the food passes and slowly moves from end to end, digestion and absorption proceeding as it goes, the undigested remnant being expelled from the anus, together with some products of the digestive tract. The food is therefore, so to speak, always outside the body, the body holding a position in relation to its food somewhat similar to that held by a plant in relation to its nutriment-containing environment.

In animals which seize living prey the mouth is provided with sharp holding or tearing teeth, or their equivalent; those which are vegetable feeders have grinding teeth or their equivalent. The former is the case with the frog and crayfish; the food of the mussel is so small and soft that it requires no such apparatus. In the latter case, by the action of the muscles of the jaws the food is torn and ground by the teeth and mixed with the saliva, which is poured out from the ducts of the salivary glands. The saliva contains an enzyme called **Ptyalin**, which is capable of converting starch into sugar, and in the mouths of such animals as have salivary glands, the digestive process begins upon any starch the food may contain. Probably a little of the sugar formed is absorbed in the mouth and passes into the circulation. The action of saliva on starch may be studied in a manner similar to that of diastase on starch, viz. with iodine and Fehling's solution.

The food is swallowed, that is, it is forced through the gullet into the stomach, where it comes in contact with the gastric juice secreted by the numerous simple peptic glands in the mucous membrane of the walls of that organ. Here it is rolled about and thoroughly mixed with the gastric juice by the contractions of the muscular walls of the stomach, and forms an even and more or less thin paste.

The gastric juice is a thin watery liquid, containing a small amount of free hydrochloric acid and of a ferment, **Pepsin,** which converts insoluble proteids into diffusible peptones. It also contains a **Rennet** ferment, which curdles milk by precipitating casein. The stomach of the calf has long been used to curdle milk in the manufacture of cheese. In the stomach, therefore, the nitrogenous proteids are brought into solution, and here also they pass to some extent into the circulation. In the stomach fats are not in general altered, but the proteid framework which holds the fat particles together is digested away and the fat set free.

From the stomach the partially digested food passes into the duodenum, where it meets with the bile and pancreatic juice.

The bile is an alkaline pigmented liquid of complex composition. It has no digestive action on proteids; in fact, the peptones produced by gastric digestion are precipitated by the bile in the small intestine, and gastric digestion is impossible in the presence of bile. It emulsifies fats to some extent, and has a powerful antiseptic action.

The pancreatic juice is a colourless alkaline liquid, which unites within itself the digestive power of all the other digestive fluids. It readily converts starch into sugar, and proteids into peptones, and first emulsifies and then decomposes fats, uniting their fatty acids with the sodium carbonate which it contains, thus forming soaps. Its proteolytic ferment, **Trypsin,** is, unlike pepsin, active only in the presence of an alkali, and there is this further and most important difference between the digestive actions of gastric and pancreatic juice on proteids, that whereas the former converts them into peptones alone, the latter further decomposes them into leucin, tyrosin, and other products of decomposition.

To the action of these juices the food in the small intestine is subjected, and the final normal result is that starches and proteids are brought into solution, and fats are emulsified or saponified.

Absorption of the Products of Digestion.—Absorption takes place to some extent in the mouth and stomach, but mainly in the small intestine. It has been already pointed out that beneath the mucous membrane of stomach and intestines the minute branches of the blood-vessels and lymphatics ramify in great numbers, and there is little difficulty in understanding that the soluble products of digestion diffuse readily into the vessels. With the emulsified fats this is not possible.

We have seen that the inner surface of the frog's intestine is thrown into thin folds and ridges which enormously increase the absorbing surface. Into these folds pass anastomosing loops of the finest branches of the blood-vessels enclosing similar loops of **Lacteals**, which are the ultimate branches of the lymphatics. They are so called because after a meal of fatty matter they are found to be full of a milky liquid containing much fat.

It is not exactly known how particles of fat pass from the intestines into the lacteals. This may take place in one of two ways. The columnar epithelial cells which line the intestine may take it up as an amœba takes up solid particles, and pass it on to the lacteals. This theory is supported by the fact that during the absorption of fat the epithelial cells contain many fat particles. Or it may be that the fine processes which project from the subjacent connective-tissue bodies between and beyond the free margins of the epithelial cells, may take up the fat and pass it on to the lacteals. Whichever is the case, the absorption is no doubt aided by the alternate shortening and lengthening of the folds of the intestine by the action of the muscle-fibres which they contain. The fat reaches the lacteals, whose contents, after passing through the glands of the lymphatics, enter the blood-circulation.

Metabolism.—The food which is absorbed from the digestive tract and the oxygen taken in by the lungs or other respiratory organ constitute the raw material from which the animal obtains matter for growth and renewal, and force for movement and heat. Constructive metabolism is slight; the material of its food is already constructed.

Concerning the final products of the destructive metabolism of the animal body there is no doubt; of the whole course of metabolism very little is known. The final products are chiefly urea, carbon dioxide, and water; the question is where these compounds are formed in the body, and what are the intermediate stages of the decompositions. It is also open to question whether they are all formed by the decomposition of protoplasm actually forming part of the living body, or whether, as appears probable from our present state of knowledge, some are not formed by the decomposition of matter taken as food, and which has never formed an integral portion of the animal.

The blood which comes from the stomach and intestines loaded with nutrient matters has to pass through the liver before reaching the systemic circulation, and the liver is the seat of great metabolic changes. First of all the bile is elaborated there and passed into the digestive tract. Secondly, the liver always contains comparatively large quantities of **Glycogen**, a carbohydrate closely allied to starch in composition and properties, and easily converted into sugar by any of the amylolytic enzymes such as diastase and ptyalin, and by dilute mineral acids. This glycogen appears to be formed and stored up in the liver, small quantities being continually converted into sugar and passed into the general circulation. Thirdly, since the liver always appears to contain urea, it appears probable that urea is formed there, not indeed out of the liver protoplasm, but out of the intermediate products of the decomposition of protoplasm, such as kreatin found in the muscles and brain, and leucin and tyrosin formed in pancreatic digestion.

The metabolism in the muscle by which energy is set free for active contraction of its fibres is not well understood. During the contraction carbon dioxide is liberated in large quantity; but since the muscle will contract when it contains no free oxygen, and in an atmosphere devoid of free oxygen, the oxidation of the substance by which energy is liberated is not directly brought about by the oxygen carried to it in the blood-circulation, but the carbon dioxide must result from the sudden decomposition of some substance which is slowly stored up in the muscle in the intervals between contraction. Whatever is the nitrogenous compound produced at the same time, it is not urea, for the amount of urea excreted during violent exercise is very little greater than during a period of rest. It is almost certainly some compound intermediate in complexity between proteids and urea, and which is afterwards further oxidized to urea in some other organ—perhaps the liver.

The Nervous System.—All the operations and movements of the body are directed and controlled by the nervous system, which sends its branches into every organ of the animal. The following short account of experiments on the frog must sufficiently demonstrate the functions and properties of the chief portions of the nervous system of this animal. Some of these the student can himself perform.

1. A frog whose cerebrum has been destroyed loses all power of initiating actions. It moves if pushed or pricked, feeds if fed, swims if placed in water, and regains a natural if placed in an unnatural position. But if left to itself it remains motionless till death. Such a frog has ceased to be automatic. Every movement made requires a stimulus from without, and every stimulus produces certain movements and no others.

2. If the optic lobes be also destroyed, the frog loses its power of balancing itself, but it can still swim and leap and perform other co-ordinated movements.

3. If the whole brain be destroyed—an operation easily performed on a chloroformed frog by severing the spinal cord close to the occipital foramen, and then passing a blunt wire into the cavity of the brain and stirring it about—the animal as an organism is virtually dead, but is still capable of co-ordinated movements. If a toe be pinched the leg is retracted. If a drop of acid be placed on its flank the hind leg is passed over the spot to rub it off. The brainless animal possesses no consciousness, and yet irritation produces co-ordinated movements as compilcated as may take place in

the conscious animal. The centre or centres for such **Reflex Actions** must be in the spinal cord. The irritation produces some change in the nerve-fibres supplied to the irritated part. This irritation is transmitted to certain nerve-cells of the cord, and as a consequence changes take place in other nerve-fibres supplied to certain muscles which cause them to contract in a manner proper to escape the irritation.

4. If the skin at the back of the thigh of a frog be cut through, and the sciatic nerve exposed and severed, pinching the toes of that leg will now produce no movement in it. If the end of the nerve still in connection with the muscles of the leg be pinched, the leg is moved, for the irritation set up in the nerve-fibres causes the muscles to contract. But no reflex action can affect this leg; the nerves transmit a stimulus, but they originate nothing.

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